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Please do not provide hits/search after 1990 (all should be 1990 and earlier).

([illegible])

[inhibitor or soluble(s) receptor] [illegible]

and

[[illegible]? or recombinant? or DNA or RNA or expres?]

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=> fil capl; d que l13; d que l16; s l13 or l16; fil wpids; d que l34
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L1          518 SEA FILE=REGISTRY ABB=ON  TUMOR NECROSIS FACTOR?/CN
L2        22744 SEA FILE=CAPLUS ABB=ON  L1 OR (TUMOR NECROSIS FACTOR) OR
                TNF
L3       286041 SEA FILE=CAPLUS ABB=ON  SOLUBLE
L4       353513 SEA FILE=CAPLUS ABB=ON  RECEPTOR#
L5         3271 SEA FILE=CAPLUS ABB=ON  L3(5A)L4
L6       528726 SEA FILE=CAPLUS ABB=ON  INHIBITOR#
L7         2343 SEA FILE=CAPLUS ABB=ON  L2(5A)(L5 OR L6)
L8       727779 SEA FILE=CAPLUS ABB=ON  CLON? OR RECOMBIN? OR DNA OR RNA
                OR ?NUCLEIC?(W)ACID#
L9          393 SEA FILE=CAPLUS ABB=ON  L7(L)L8
L11         966 SEA FILE=CAPLUS ABB=ON  L5 NOT (1991-1997)/PY
L13           6 SEA FILE=CAPLUS ABB=ON  L9 AND L11


L1          518 SEA FILE=REGISTRY ABB=ON  TUMOR NECROSIS FACTOR?/CN
L2        22744 SEA FILE=CAPLUS ABB=ON  L1 OR (TUMOR NECROSIS FACTOR) OR
                TNF
L3       286041 SEA FILE=CAPLUS ABB=ON  SOLUBLE
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L5         3271 SEA FILE=CAPLUS ABB=ON  L3(5A)L4
L6       528726 SEA FILE=CAPLUS ABB=ON  INHIBITOR#
L7         2343 SEA FILE=CAPLUS ABB=ON  L2(5A)(L5 OR L6)
L11         966 SEA FILE=CAPLUS ABB=ON  L5 NOT (1991-1997)/PY
L15         380 SEA FILE=CAPLUS ABB=ON  L7(L)EXPRESS?
L16           5 SEA FILE=CAPLUS ABB=ON  L15 AND L11


L35           8 L13 OR L16


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MOST RECENT DERWENT WEEK             9717   <199717/DW>
DERWENT WEEK FOR CHEMICAL CODING:    9709
DERWENT WEEK FOR POLYMER INDEXING:   9714
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L17         1225 SEA FILE=WPIDS ABB=ON  (TUMOR OR TUMOUR)(W)NECROSIS(W)FAC
                 TOR OR TNF
L18          183 SEA FILE=WPIDS ABB=ON  SOLUBLE(5A)RECEPTOR#
L19        45462 SEA FILE=WPIDS ABB=ON  INHIBITOR#
L21         1000 SEA FILE=WPIDS ABB=ON  L17 NOT 1991/PY
L22          965 SEA FILE=WPIDS ABB=ON  L17 NOT 1992/PY
L23          924 SEA FILE=WPIDS ABB=ON  L17 NOT 1993/PY
L24          903 SEA FILE=WPIDS ABB=ON  L17 NOT 1994/PY
L25          828 SEA FILE=WPIDS ABB=ON  L17 NOT 1995/PY
L26          772 SEA FILE=WPIDS ABB=ON  L17 NOT 1996/PY
L27         1088 SEA FILE=WPIDS ABB=ON  L17 NOT 1997/PY
L28         1224 SEA FILE=WPIDS ABB=ON  (L21 OR L22 OR L23 OR L24 OR L25 O
                 R L26 OR L27)
L29          168 SEA FILE=WPIDS ABB=ON  L17(5A)(L18 OR L19)
L30        30277 SEA FILE=WPIDS ABB=ON  CLON? OR RECOMBIN? OR DNA OR RNA O
                 R DEOXYRIBONUCLEIC OR RIBONUCLEIC
L31        33336 SEA FILE=WPIDS ABB=ON  EXPRESS?
L34            8 SEA FILE=WPIDS ABB=ON  L29(10A)(L30 OR L31) AND L28

=> dup rem l35,l34
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PROCESSING COMPLETED FOR L34
L36           16 DUP REM L35 L34 (0 DUPLICATES REMOVED)

=> d bib ab l36 1-16; fil hom

L36  ANSWER 1 OF 16  WPIDS   COPYRIGHT 1997 DERWENT INFORMATION LTD
AN   97-154258 [14]   WPIDS
DNN  N97-127417       DNC C97-049367
TI   Monoclonal antibodies against soluble TNF-alpha receptors
     p55 and p75 - as well as against TNF-alpha and its
     analogues, also new stable hybridoma cell lines.
DC   B04 D16 S03
IN   CURIN, SERBEC V; GADERC, POREKAR V; MENART, V; OCVIRK, J;
     SCHUURBIERS, A; STABUC, B; STALC, A; ZUNEC, P
PA   (LEKT) LEK TOVARNA FARM KEMICNIH; (REPU-N) ZAVOD REPUBLIKE SLOVENIJE
     ZA TRANSFUZIJO
CYC  70
PI   WO 9706251  A1 970220 (9714)* EN   36 pp
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RW: AT BE CH DE DK EA ES FI FR GB GR IE IT KE LS LU MC MW NL OA PT SD SE SZ UG
W: AL AM AT AU AZ BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IL IS JP KE KG KP KR KZ LK LR LS LT LU LV MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SK TJ TM TR TT UA UG US UZ VN

ADT WO 9706251 A1 WO 96-SI18 960731

PRAI SI 96-206 960628; SI 95-249 950804

AB WO 9706251 A UPAB: 970407

Monoclonal antibodies (I) and their fragments, that bind to the surface area of human **recombinant TNF**- alpha , **TNF**- alpha analogues, both **soluble TNF**- alpha **receptors** p55 and p75, and complexes of these molecules, are new. Also claimed are: (1) stable hybridoma cell lines capable of producing (I) or their fragments; (2) **TNF**- alpha analogue Cys95His107His108Cys148 (II).

USE - (I) are useful for diagnosis, prophylaxis and treatment of diseases related to an increased amt. of **TNF**- alpha (claimed), e.g. septic shock, AIDS, cerebral malaria, graft vs. host disease, rheumatoid arthritis. They may be used in kits for determn. of human **TNF**- alpha , free and complexed with soluble **TNF**- alpha receptors p55 and p57, as well as of **TNF**- alpha analogues in biological samples. (I) can be used in a formulation opt. with antihistaminics or glucocorticoids.

ADVANTAGE - Formulations of (I) enable determn. both of free receptors p55 and p75 and **TNF**- alpha and of complexes of the 2 soluble **TNF**- alpha receptors with **TNF**- alpha or its analogues, which has not previously been possible.

Dwg.0/1

L36 ANSWER 2 OF 16 WPIDS COPYRIGHT 1997 DERWENT INFORMATION LTD

AN 96-333771 [33] WPIDS

DNC C96-105401

TI Treatment of acute pancreatitis - using **tumour necrosis factor** antagonist opt. combined with interleukin-1 receptor antagonist.

DC B04

IN NORMAN, J G

PA (UYSF-N) UNIV SOUTH FLORIDA

CYC 19

PI WO 9620729 A1 960711 (9633)* EN 47 pp

RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
W: CA JP MX

ADT WO 9620729 A1 WO 96-US107 960102

PRAI US 95-369386 950106

AB WO 9620729 A UPAB: 960823

Treatment of acute pancreatitis comprises admin. of a **tumour necrosis factor** (**TNF**) antagonist or its salt.

USE - **Recombinant tumour necrosis factor soluble receptor** (rTNFsr) and Interleukin-1 **receptor** antagonist (IL-1ra) can be administered e.g. i.v., i.a., i.m., i.p., i.n. and by implant, pref. i.v. at 100 ng-100 mg/kg/day rTNFsr and IL-1ra. rTNFsr is pref. administered alone i.v. at 2-5 mg/kg in a total vol. of 10 ml followed by a daily i.v. injection of 2-5 mg/kg/day in a total vol. of 10 ml or a continuous infusion allowing sufficient levels of

rTNFsr to be maintained (0.5mg/kg/hr) (all claimed).
Dwg.10/10

L36 ANSWER 3 OF 16 WPIDS COPYRIGHT 1997 DERWENT INFORMATION LTD
AN 92-284678 [34] WPIDS
DNC C92-126670
TI Use of **tumour necrosis factor** inhibitory proteins - for treating and preventing **TNF** -mediated diseases e.g. adult respiratory distress syndrome, pulmonary fibrosis, arthritis, etc..
DC B04 D16
IN CARMICHAEL, D F; KOHNO, T; RUSSELL, D; SMITH, C G; THOMPSON, R C; RUSSEL, D
PA (SYND) SYNERGEN INC
CYC 35
PI WO 9213095 A1 920806 (9234)* EN 65 pp
RW: AT BE CH DE DK ES FR GB GR IT LU MC NL OA SE
W: AT AU BB BG BR CA CH CS DE DK ES FI GB HU JP KP KR LK LU MG MW NL NO PL RO RU SD SE
AU 9212356 A 920827 (9247)
EP 567566 A1 931103 (9344) EN
R: AT BE CH DE DK ES FR GB GR IT LI LU MC NL SE
NO 9302610 A 930909 (9348)
JP 06506446 W 940721 (9433) 20 pp
EP 567566 A4 941005 (9534)
AU 9642288 A 960418 (9623)
ADT WO 9213095 A1 WO 92-US432 920117; AU 9212356 A AU 92-12356 920117, WO 92-US432 920117; EP 567566 A1 EP 92-904429 920117, WO 92-US432 920117; NO 9302610 A WO 92-US432 920117, NO 93-2610 930719; JP 06506446 W JP 92-504597 920117, WO 92-US432 920117; EP 567566 A4 EP 92-904429 ; AU 9642288 A Div ex AU 92-12356 920117, AU 96-42288 960202
FDT AU 9212356 A Based on WO 9213095; EP 567566 A1 Based on WO 9213095; JP 06506446 W Based on WO 9213095
PRAI US 91-644345 910118
AB WO 9213095 A UPAB: 931025
Treating or preventing **tumour necrosis factor** (**TNF**) mediated diseases comprises administering to a patient a therapeutically effective amt. of a **TNF** inhibitor.

The **TNF** inhibitor is 30 kD or 40 kD **TNF**. The 40 kD inhibitor may be the full-length 40 kD **TNF** inhibitor, 40 kD **TNF** inhibitor delta 51 to 40 kD **TNF** inhibitor delta 53. It may also be a mutein of the 30 kD **TNF** inhibitor, e.g. C105 30 kD **TNF** inhibitor or a modified protein, e.g. 30 kD **TNF** inhibitor-PEG or a C105-PEG 3400 dB (dumbbell). Pref. **TNF inhibitor** (binding protein or receptor) prodn. is by **recombinent DNA** technology, as it is capable of producing comparatively higher amts. of **TNF** inhbitors or greater purities.

USE/ADVANTAGE - Adult respiratory distress syndrome, pulmonary fibrosis, arthritis, septic shock and inflammatory bowel disease may be treated or prevented. The **TNF** inhibitor is administered in a pharmaceutically acceptable carrier, pref. in a liq. form.

Dosage for **TNF** mediated septic shock is esp. 1-50 mg/kg/24 hrs. equally administered every 3 hrs.

Dwg.0/17

L36 ANSWER 4 OF 16 WPIDS COPYRIGHT 1997 DERWENT INFORMATION LTD
AN 91-218455 [30] WPIDS
DNN N91-166610 DNC C91-094893
TI Judging infantile acute febrile muco-cutaneous lymph node syndrome - comprises determn. of **tumour necrosis factor** inhibitor activity in human urine.
DC B04 P31 S03
PA (TEIJ) TEIJIN LTD
CYC 1
PI JP 03139294 A 910613 (9130)*
ADT JP 03139294 A JP 89-276133 891025
PRAI JP 89-276133 891025
AB JP03139294 A UPAB: 930928
Judging the state of MCLS comprises determining **TNF** (**tumour necrosis factor**) inhibitor activity in human urine.

The amt. of **TNF** inhibitor is determined by the addn. of a given amt. of urine sample to the system, which utilises the system of examining the effect of killing cells of **TNF** to tumour cell. Based on that **TNF** inhibitor activity is present in high rate in urine of MCLS patient and strong **TNF** inhibitor activity appears in urine at fever period before the formation of coronary aneurysm. The **TNF inhibitor** activity is **expressed** by **TNF** inhibition rate by the following equation.

TNF inhibition rate = ((OD595)TNFfree - (OD595) **TNF** with sample) / ((OD595)TNFfree - (OD595)**TNF** without sample) x 100%.

When more than 20% of **TNF** inhibition rate in urine lasts for 3-6 days, the possibility of causing a morbid change of the coronary arteries is high. Esp., a morbid change of the coronary arteries is apt to appear in 4-7 days after lasting of 3-6 days of the high inhibition rate.

USE/ADVANTAGE - The dangerous state of MCLS can be forseen before the occurrence of a morbid change of the coronary arteries, consequently suitable remedy or treatment can be taken for the alleviation of fever by dosage of aspirin or remedy by dosage of globulin, etc..
0/0

L36 ANSWER 5 OF 16 WPIDS COPYRIGHT 1997 DERWENT INFORMATION LTD
AN 91-203636 [28] WPIDS
DNC C91-088232
TI Antitumoural compsn. - contains active component of **tumour necrosis factor** and acidosis accelerator selected from glucose and/or lactose.
DC B04 D16
PA (TEIJ) TEIJIN LTD
CYC 1
PI JP 03127741 A 910530 (9128)*
ADT JP 03127741 A JP 89-264762 891011
PRAI JP 89-264762 891011
AB JP03127741 A UPAB: 930928
Compsn. contains (1) **Tumour Necrosis**

Factor (**TNF**) and (2) acidosis accelerator.
The acidosis accelerator is pref. glucose and/or lactose. The **TNF** includes **recombinant** human-**TNF** (rHu-**TNF**). The **inhibitors** contain 1x10power4-5x10power5 U/m2/hr., in the form of an injection prepn., suppository, spray, capsule or tablets.
USE/ADVANTAGE - For treating malignant tumour.
0/0

L36 ANSWER 6 OF 16 WPIDS COPYRIGHT 1997 DERWENT INFORMATION LTD
AN 91-186774 [26] WPIDS
DNC C91-080814
TI Recombinant **tumour necrosis factor** binding protein I - prepd. by transfecting eukaryotic cells with vector contg. **deoxyribonucleic** acid encoding human type I **TNF receptor** or **soluble** domain.
DC B04 D16
IN ADERKA, D; BRAKEBUSCH, C; ENGELMANN, H; KEMPER, O; NOPHAR, Y; WALLACH, D
PA (WALL-I) WALLACH D; (YEDA) YEDA RES & DEV CO LTD
CYC 19
PI EP 433900 A 910626 (9126)*
R: AT BE CH DE ES FR GB GR IT LI LU NL SE
AU 9068037 A 910620 (9132)
CA 2032191 A 910614 (9134)
ZA 9010036 A 911030 (9149)
JP 04299989 A 921023 (9249) 35 pp
JP 05078396 A 930330 (9317) 21 pp
AU 642938 B 931104 (9351)
EP 433900 B1 950920 (9542) EN 30 pp
R: AT BE CH DE DK ES FR GB GR IT LI LU NL SE
DE 69022559 E 951026 (9548)
ES 2080098 T3 960201 (9612)
IL 92697 A 960331 (9622)
ADT EP 433900 A EP 90-124133 901213; ZA 9010036 A ZA 90-10036 901213; JP 04299989 A JP 90-419119 901226; JP 05078396 A JP 90-419240 901213; AU 642938 B AU 90-68037 901213; EP 433900 B1 EP 90-124133 901213; DE 69022559 E DE 90-622559 901213, EP 90-124133 901213; ES 2080098 T3 EP 90-124133 901213; IL 92697 A IL 89-92697 891213
FDT AU 642938 B Previous Publ. AU 9068037; DE 69022559 E Based on EP 433900; ES 2080098 T3 Based on EP 433900
PRAI IL 89-92697 891213; IL 90-95064 900712
AB EP 433900 A UPAB: 930928
Prodn. of a sol. recombinant protein, selected from human **Tumour Necrosis Factor** Binding protein I (TBP-I), biologically active precursors and analogues, comprises: (a) transfecting eucaryotic cells with an expression vector comprising a DNA molecule (I) encoding the whole human type I **TNF** receptor culturing the transfected cells to secrete the protein into the medium. Also claimed are the sol. protein, selected from precursors and analogues of TBP-I.
USE/ADVANTAGE - TBP-I produced is the sol. form of type I **TNF**-receptor and constitutes a fragment of the cell surface form of this receptor, corresp. to its extracellular domain. The **TNF**-receptor plays a key role in initiation and control of the cellular response to the cytokines IL-1 and IL-6. TBP-I is

produced in large amts.. @(30pp Dwg.No.0/6)@

L36 ANSWER 7 OF 16 WPIDS COPYRIGHT 1997 DERWENT INFORMATION LTD
AN 91-081851 [12] WPIDS
DNC C91-034797
TI Insoluble **tumour necrosis factor** binding proteins - and DNA encoding them, useful in pharmaceutical prods. and for antibody prodn..
DC B04 D16
IN BROCKHAUS, M; DEMBIC, Z; GENTZ, R; LESSLAUER, W; LOTSCHER, H; SCHLAEGER, E J; SCHLAEGER, E
PA (HOFF) HOFFMANN-LA ROCHE AG; (HOFF) HOFFMANN LA ROCHE & CO AG F; (HOFF) HOFFMANN LA ROCHE INC
CYC 2
PI EP 417563 A 910320 (9112)*
JP 04164099 A 920609 (9229) 21 pp
EP 417563 A3 920429 (9329)
US 5610279 A 970311 (9716) 20 pp
ADT EP 417563 A EP 90-116707 900831; JP 04164099 A JP 90-240176 900912; EP 417563 A3 EP 90-116707 900831; US 5610279 A Cont of US 90-580013 900910, US 93-95640 930721
PRAI CH 89-3319 890912; CH 90-746 900308; CH 90-1347 900420
AB EP 417563 A UPAB: 931116
Insoluble **TNF**-BPs, or soluble and insoluble fragments of these (55 or 75 kD-SDS PAGE), in homogeneous form, and their salts are new. DNA sequences (I) encoding **TNF**-BPs, and derived amino acid sequences are given in the specification. (I) consists of a sequence encoding a **TNF**-BP and a sequence encoding all domains, except the first domain, of the human Ig having chain constant region (IgG, IgA, IgM, IgE, esp. IgM, IgG, type 1 or 3). **TNF**-BP can be prepared by culturing hosts, e.g. mammalian or insect cells, transformed with a vector containing (I), and isolating and purifying the product.

USE/ADVANTAGE - **TNF**-BP is used in a therapeutic product, and as an antigen for the production of mono- and polyclonal antibodies.

In an example specific **TNF**-binding capacity at various concns. is measured in a filter test: Kd = 10 power(-9)-10 power(-10) M. @(26pp Dwg.No.0/4)
0/4

L36 ANSWER 8 OF 16 CAPLUS COPYRIGHT 1997 ACS
AN 1991:222818 CAPLUS
DN 114:222818
TI Cloning and expression of a cDNA for tumor necrosis factor receptor
IN Hauptmann, Rudolf; Himmler, Adolf; Maurer-Fogy, Ingrid; Stratowa, Christian
PA Boehringer Ingelheim International G.m.b.H., Fed. Rep. Ger.
SO Eur. Pat. Appl., 51 pp.
CODEN: EPXXDW
PI EP 393438 A2 901024
DS R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE
AI EP 90-106624 900406
PRAI DE 89-3913101 890421
DE 89-3920282 890621
DT Patent

LA German
AB CDNAs encoding the receptor for tumor necrosis factor are cloned and expressed to manuf. the **receptor** and a **sol.** analog. The protein was purified from urine of uremic patients by affinity chromatog. using immobilized tumor necrosis factor as affinity ligand, in addn. to ion-exchange and reverse-phase chromatog. Sequencing of peptides was used to identify oligonucleotides for use as probes or as primers for polymerase chain reaction for cDNA synthesis. These probes were then used to identify the relevant cDNA clone from a library prepd. from a tumor necrosis factor-stimulated human fibrosarcoma cell line HS913T. Expression vectors using viral promoters to express the cDNA for the intact or sol. form of the protein in animal cells were then constructed. Transient expression assays for manuf. of the sol. form in COS-7 cells yielded 146 ng receptor/mL culture supernatant from 106 cells after 72 h for the most efficient construct.

L36 ANSWER 9 OF 16 CAPLUS COPYRIGHT 1997 ACS
AN 1991:205224 CAPLUS
DN 114:205224
TI A second tumor necrosis factor receptor gene product can shed a naturally occurring tumor necrosis factor inhibitor
AU Kohno, Tadahiko; Brewer, Michael T.; Baker, Susan L.; Schwartz, Phillip E.; King, Michael W.; Hale, Karin K.; Squires, Charles H.; Thompson, Robert C.; Vannice, James L.
CS Synergen, Inc., Boulder, CO, 80301, USA
SO Proc. Natl. Acad. Sci. U. S. A. (1990), 87(21), 8331-5
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB An **inhibitor** of **tumor necrosis factor** (**TNF**) has been isolated from the human histiocytic lymphoma cell line U-937 that is capable of inhibiting both TNF-.alpha. and TNF-.beta.. Protein sequencing has verified that it is distinct from a previously described **TNF inhibitor** that is a **sol.** fragment of a **TNF receptor** mol. (TNFrI). The cDNA sequence of this second **TNF inhibitor clone** suggests that it is also a **sol.** fragment of a **TNF receptor** . **Expression** of this cDNA sequence in COS-7 cells verified that it encodes a receptor for TNF-.alpha. (TNFrII) that can give rise to a sol. **inhibitor** of **TNF** -.alpha., presumably through proteolytic cleavage. The extracellular domain of TNFrII has significant homol. with that of TNFrI and these two receptors share a striking conservation of cysteine residue alignment with the extracellular domain of the nerve growth factor receptor. These three receptor mols. are therefore members of a family of polypeptide hormone receptors.

L36 ANSWER 10 OF 16 CAPLUS COPYRIGHT 1997 ACS
AN 1991:158076 CAPLUS
DN 114:158076
TI **Cloning** of human **tumor necrosis factor** (**TNF**) **receptor** cDNA and **expression** of **recombinant soluble** **TNF**-binding protein

AU Gray, Patrick W.; Barrett, Kathy; Chantry, David; Turner, Martin; Feldmann, Marc
CS Charing Cross Sunley Res. Cent., Hammersmith/London, W6 8LW, UK
SO Proc. Natl. Acad. Sci. U. S. A. (1990), 87(19), 7380-4
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB The cDNA for one of the receptors for human tumor necrosis factor (TNF) has been isolated. This cDNA encodes a protein of 455 amino acids that is divided into an extracellular domain of 171 residues and a cytoplasmic domain of 221 residues. The extracellular domain has been engineered for expression in mammalian cells, and this recombinant deriv. binds TNF.alpha. with high affinity and inhibits its cytotoxic activity in vitro. The TNF receptor exhibits similarity with a family of cell surface proteins that includes the nerve growth factor receptor, the human B-cell surface antigen CD40, and the rat T-cell surface antigen OX40. The TNF receptor contains 4 cysteine-rich subdomains in the extracellular portion. Mammalian cells transfected with the entire TNF receptor cDNA bind radiolabeled TNF.alpha. with an affinity of 2.5 .times. 10-9 M. This binding can be competitively inhibited with unlabeled TNF.alpha. or lymphotoxin (TNF.beta.).

L36 ANSWER 11 OF 16 CAPLUS COPYRIGHT 1997 ACS
AN 1990:495927 CAPLUS
DN 113:95927
TI Characterization of a tumor necrosis factor .alpha. (TNF-.alpha.) inhibitor: evidence of immunological cross-reactivity with the TNF receptor
AU Seckinger, Philippe; Zhang, Jian Hua; Hauptmann, Bettina; Dayer, Jean Michel
CS Dep. Med., Hop. Cantonal Univ., Geneva, 1211, Switz.
SO Proc. Natl. Acad. Sci. U. S. A. (1990), 87(13), 5188-92
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB Previous studies have shown that urine of febrile patients contains a tumor necrosis factor .alpha. inhibiting activity (TNF-.alpha. Inh) when tested in a cytotoxicity assay using the tumor necrosis factor .alpha. (TNF-.alpha.)-susceptible cell line L929. In the present study, the relationship between the **TNF**-.alpha. Inh and a potential **sol**. form of the **receptor** was investigated as the former has been shown to block TNF-.alpha. activities by binding to the ligand. Human TNF-.alpha. is affected to a greater extent than is murine TNF-.alpha.. This species specificity of the inhibitor correlates with the binding studies of TNF receptor interactions already reported. A polyclonal antibody was raised to TNF-.alpha. Inh that neutralizes its activity and does not recognize TNF-.alpha.. Solubilized cross-linked 125I-labeled TNF-.alpha. receptor complex could be immunopptd. by using either anti-TNF-.alpha. or anti-TNF-.alpha. Inh antibody, suggesting immunol. cross-reactivity between the receptor and the inhibitor. By using fluorescein isothiocyanate-coupled TNF-.alpha., it was possible to visualize by fluorescence-activated cell sorter anal. the TNF-.alpha. receptor on phytohemagglutiin/interleukin 2-activated T cells. A similar increase of immunofluorescence

intensity of the activated T cells was obsd. by using anti-TNF-.alpha. Inh antibody revealed with a fluorescein isothiocyanate-coupled goat anti-rabbit IgG1 conjugate, suggesting that the TNF-.alpha. Inh is also **expressed** as a membrane protein. The TNF-.alpha. Inh originally described might be a **sol**. form of the **TNF receptor** itself.

L36 ANSWER 12 OF 16 CAPLUS COPYRIGHT 1997 ACS
AN 1991:200676 CAPLUS
DN 114:200676
TI **Soluble** forms of **tumor necrosis factor receptors** (**TNF**-Rs). The cDNA for the type I TNF-R, **cloned** using amino acid sequence data of its soluble form, encodes both the cell surface and a **soluble** form of the **receptor**
AU Nophar, Yaron; Kemper, Oliver; Brakebusch, Cord; Engelmann, Hartmut; Zwang, Raya; Aderka, Dan; Holtmann, Helmut; Wallach, David
CS Dep. Mol. Genet. Virol., Weizmann Inst. Sci., Rehovot, 76100, Israel
SO EMBO J. (1990), 9(10), 3269-78
CODEN: EMJODG; ISSN: 0261-4189
DT Journal
LA English
AB Two proteins which specifically bind tumor necrosis factor (TNF) have recently been isolated from human urine. The 2 proteins cross-react immunol. with 2 species of cell surface TNF receptors (TNF-R). Antibodies against 1 of the 2 TNF-binding proteins (TBPI) were found to have effects characteristic of TNF, including stimulating phosphorylation of specific cellular proteins. Oligonucleotide probes designed on the basis of the NH2-terminal amino acid sequence of TBPI were used to clone the cDNA for the structurally related cell surface type 1 TNF-R. The extracellular domain of the receptor is composed of 4 internal cysteine-rich repeats, homologous to structures repeated 4 times in the extracellular domains of the nerve growth factor receptor and the B-lymphocyte surface antigen CDw40. The amino acid compn. and size of the extracellular domain of the type I TNF-R closely resemble those of TBPI. The COOH-terminal amino acid sequence of the 4 cysteine rich repeats within the extracellular domain of the type I TNF-R matches the COOH-terminal sequence of TBPI. Amino acid sequences in the extracellular domain also fully match other sequences found in TBPI. On the other hand, amino acid sequences in the sol. form of the type II TNF-R (TBPII), while indicating a marked homol. of structure, did not suggest any identity between this protein and the extracellular domain of the type I TNF-R. CHO cells transfected with type I TNF-R cDNA produced both cell surface and **sol**. forms of the **receptor**. The receptor produced by CHO cells was recognized by several monoclonal antibodies against TBPI, reacting with several distinct epitopes in this mol. Thus, the sol. forms of the TNF-Rs are structurally identical to the extracellular cytokine-binding domains of these **receptors** and the **sol**. forms are, at least partly, derived from the same transcripts that encode the cell surface receptors.

L36 ANSWER 13 OF 16 CAPLUS COPYRIGHT 1997 ACS
AN 1991:465733 CAPLUS

DN 115:65733
TI Molecular **cloning** and **expression** of human and rat **tumor necrosis factor** **receptor** chain (p60) and its **soluble** derivative, **tumor necrosis factor**-binding protein
AU Himmler, Adolf; Maurer-Fogy, Ingrid; Kroenke, Martin; Scheurich, Peter; Pfizenmaier, Klaus; Lantz, Mikael; Olsson, Inge; Hauptmann, Rudolf; Stratowa, Christian; Adolf, Guenther R.
CS Ernst Boehringer Inst., Bender and Co. G.m.b.H., Vienna, 1121, Austria
SO DNA Cell Biol. (1990), 9(10), 705-15
CODEN: DCEBE8; ISSN: 1044-5498
DT Journal
LA English
AB Tumor necrosis factor-.alpha.), a protein released by activated macrophages, is involved in a wide variety of human diseases including septic shock, cachexia, and chronic inflammation. TNF binding protein (TNF-BP), a glycoprotein with high affinity to TNF-A isolated from urine, acts as an **inhibitor** of **TNF** -.alpha. by competing with the cell-surface TNF receptor. This report describes the partial amino acid sequencing of human TNF-BP as well as the isolation, sequence, and **expression** of cDNA **clones** encoding a human and rat TNF receptor. The calcd. Mr of the mature human and rat TNF receptor chains is 47,526 and 48,072, resp. The extracellular ligand binding domain represents the sol. TNF-BP which is released by proteolytic cleavage. TNF-BP contains 24 cysteine residues and three potential N-glycosylation sites and shows sequence homol. to the extracellular portions of TNF-R p80 chain and nerve growth factor receptor. Transfection of the human TNF receptor cDNA into mammalian cells resulted in increased binding capacity for TNF-.alpha. and increased reactivity with a monoclonal antibody directed against the human TNF receptor chain p60. When a stop codon was introduced into the cDNA at the site corresponding to the carboxyl terminus of TNF-BP, transfected cells secreted a protein that reacted with antibodies raised against natural TNF-BP.

L36 ANSWER 14 OF 16 CAPLUS COPYRIGHT 1997 ACS
AN 1991:556746 CAPLUS
DN 115:156746
TI Infusion of tumor necrosis factor (TNF) causes an increase in circulating TNF-binding protein in humans
AU Lantz, Mikael; Malik, Saleem; Slevin, Maurive L.; Olsson, Inge
CS Res. Dep., Lund Hosp., Lund, S-22185, Swed.
SO Cytokine (Philadelphia) (1990), 2(6), 402-6
CODEN: CYTIE9; ISSN: 1043-4666
DT Journal
LA English
AB Serum samples from cancer patients receiving i.v. infusions of **recombinant** tumor necrosis factor (rTNF) and **recombinant** interferon-.gamma. (rIFN-.gamma.) were analyzed for TNF and the TNF-binding protein (TNF-BP). **TNF**-BP is a **sol.** fragment of the transmembrane **TNF** **receptor** with antagonistic effects to **TNF** and is released by proteolytic cleavage of the receptor. During a 60-min infusion of rTNF, peak serum levels of rTNF were obsd. after 30-60

min, and a transient increase of circulating TNF-BP was obsd. with peak levels between 30 and 120 min. Injection of IFN-.gamma. alone did not affect the levels of TNF and TNF-BP. Thus, administration of rTNF leads to release into the circulation of TNF-BP, which may modulate both systemic and local effects of TNF and influence its therapeutic efficacy.

L36 ANSWER 15 OF 16 CAPLUS COPYRIGHT 1997 ACS
AN 1990:513538 CAPLUS
DN 113:113538
TI Purification of **soluble** cytokine **receptors** from normal human urine by ligand-affinity and immunoaffinity chromatography
AU Novick, Daniela; Engelmann, Hartmut; Wallach, David; Leitner, Orit; Revel, Michel; Rubinstein, Menachem
CS Dep. Mol. Genet. Virol., Weizmann Inst. Sci., Rehovot, 76100, Israel
SO J. Chromatogr. (1990), 510, 331-7
CODEN: JOCRAM; ISSN: 0021-9673
DT Journal
LA English
AB Affinity chromatog. of crude human urinary proteins on either human **recombinant** interleukin-6 (rL-6) or human **recombinant** interferon-.gamma. (rIFN-.gamma.) or anti IFN-.gamma. receptor (IFN-.gamma.-R) monoclonal antibodies (McAb) yielded the two resp. **sol. receptors** in significant amts. A single sequence of 30 amino acid residues was obtained by N-terminal microsequencing of the protein peak purified in tandem by affinity chromatog. on an IL-6 column and reversed-phase HPLC. This sequences was identical with the predicted N-terminal sequence of IL-6-R as previously reported. The purified IL-6-R retained its biol. activity. It was used for the prepn. of specific anti IL-6-R monoclonal antibodies. Anal. of the eluted proteins from both IFN-.gamma. and anti IFN-.gamma.-R columns by inhibition of solid-phase RIA, ELISA, SPS-PAGE and Western blotting proved the existence of sol. IFN-.gamma.-R in normal urine. This finding together with the already known presence of **sol. TNF receptors** and a **sol.** IL-2 **receptor** found both in plasma and in urine indicates that release of **sol.** cytokine **receptors** into body fluids is a general phenomenon which occurs under normal physiol. conditions.

L36 ANSWER 16 OF 16 WPIDS COPYRIGHT 1997 DERWENT INFORMATION LTD
AN 89-087746 [12] WPIDS
CR 90-350112 [47]; 91-045654 [07]; 92-374839 [46]
DNC C89-038816
TI **Tumour Necrosis Factor** inhibitory protein - isolated from urine and having ability to inhibit the binding of **TNF** to its receptors and its cytotoxic effect.
DC B04 D16 S03
IN ADERKA, D; ENGELMANN, H; RUBINSTEIN, M; WALLACH, D
PA (YEDA) YEDA RES & DEV CO LTD
CYC 18
PI EP 308378 A 890322 (8912)* EN 19 pp
R: AT BE CH DE ES FR GB GR IT LI LU NL SE
AU 8822068 A 890316 (8924)

JP 02000200 A 900105 (9007)
ZA 8806818 A 900131 (9009)
EP 308378 B1 941130 (9501) EN 26 pp
R: AT BE CH DE ES FR GB GR IT LI LU NL SE
DE 3852255 G 950112 (9507)
ES 2067486 T3 950401 (9520)
IL 83878 A 950731 (9540)
US 5512544 A 960430 (9623) 5 pp
ADT EP 308378 A EP 88-830365 880913; JP 02000200 A JP 88-228307 880912; ZA 8806818 A ZA 88-6818 880913; EP 308378 B1 EP 88-830365 880913; DE 3852255 G DE 88-3852255 880913, EP 88-830365 880913; ES 2067486 T3 EP 88-830365 880913; IL 83878 A IL 87-83878 870913; US 5512544 A Cont of US 88-243092 880912, CIP of US 90-524263 900516, CIP of US 92-876828 920430, US 92-879373 920507
FDT DE 3852255 G Based on EP 308378; ES 2067486 T3 Based on EP 308378
PRAI IL 87-83878 870913; IL 89-90339 890518; IL 91-98078 910507
AB EP 308378 A UPAB: 960618
A **Tumour Necrosis Factor** (**TNF**) inhibitory protein, salts, Functional derivs. and active fractions and mixts. of any of these having the ability to inhibit (a) the binding of **TNF** to its receptors and (b) the cytotoxic effect of **TNF** are claimed.

Also claimed is A **DNA** molecule comprising the nucleotide sequence coding for the **TNF inhibitory** protein, a replicable **expression** vehicle contg. the **DNA** molecule and a host cell transformed with the replicable expression vehicle.

USE - The **TNF** inhibitory protein can be used for antagonising the deleterious effects of **TNF** in mammals, e.g. for treating conditions where there is an over prodn. of endogenous **TNF**, such as in cases of septic shock, cachexia; graft-versus-host reactions or autoimmune diseases like rheumatiod arthritis. It can also be used in cases of **TNF** intoxication caused by exogenous administration of excessive amts of **TNF**.

0/6
Dwg.0/6

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show files
File 155:MEDLINE(R) 1966-1997/Jun W3
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         (c)1997 RoySocChm,DECHEMA,FizChemie
File 358:Current BioTech Abs 1983-1997/May
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Set     Items   Description
S1      96897   (TUMOR OR TUMOUR)(W)NECROSIS(W)FACTOR OR TNF
S2    1098976   INHIBITOR? OR (SOLUBLE(5N)RECEPTOR?)
S3     113545   EXPRESS
S4       6390   S1(5N)S2
S5     364426   RECOMBIN?
S6    1195237   DNA
S7     628600   RNA
S8      48595   RIBONUCLEIC OR DEOXYRIBONUCLEIC
S9     539775   CLON?
S10      6328   S4 NOT (1991 OR 1992 OR 1993 OR 1994 OR 1995 OR 1996 OR 19-
            97)/PY
S12       739   S4(10N)(S5-S9)
S13       727   S12 AND S10
S14         3   S4(5N)(S3 OR S5-S9) AND S10
S15       147   S4(S)(S3 OR S5-S9) AND S10
S16        63   RD (unique items)
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 16/7/1     (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
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09038515   97236467
 Soluble interleukin-2 receptor serum level as a predictor of locoregional
control  and survival for patients with head and neck carcinoma: results of
a multivariate prospective study.
  Tartour E; Deneux L; Mosseri V; Jaulerry C; Brunin F; Point D; Validire P
; Dubray B; Fridman WH; Rodriguez J
  Head and Neck Oncology Group, Institut Curie, Paris, France.
  Cancer (UNITED STATES)   Apr 1 1997,  79 (7) p1401-8,  ISSN 0008-543X
Journal Code: CLZ
  Languages: ENGLISH
  Document type: JOURNAL ARTICLE
  BACKGROUND: The diagnosis and follow-up of head and neck carcinoma
patients are based exclusively on clinical staging, which cannot always
predict clinical outcome accurately. Because oral squamous cell carcinomas
produce interleukin (IL)-6 and tumor necrosis factor (TNF)-alpha and
*express* IL-2 receptors, the authors assessed the prognostic value of the
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serum levels of these markers. METHODS: Serum levels of IL-6, *TNF*-alpha, *soluble* IL-2 *receptors* (s-IL-2-R), and acute phase proteins were measured at the time of diagnosis in a prospective study of 85 patients with primary squamous cell carcinoma of the head and neck. The influence of each clinical and laboratory parameter on locoregional control and survival was analyzed. RESULTS: At presentation, a relationship was observed between advanced tumor (T) classification and high serum levels of CRP ($P = 0.0015$) and s-IL-2-R ($P < 0.05$). A high lymph node (N) classification was significantly associated with elevated serum IL-6 ($P = 0.01$) and CRP levels ($P = 0.0002$). In the univariate analysis, T classification, N classification, performance status, Prognostic Inflammatory and Nutritional Index, and serum s-IL-2-R level were significantly correlated with both locoregional control and survival. Multivariate analysis showed that the only significant prognostic factors related independently to locoregional control were N classification ($P = 0.02$) and serum s-IL-2-R level ($P = 0.02$). In a Cox multivariate analysis, serum s-IL-2-R level was found to be the most predictive factor of survival ($P = 0.0001$). CONCLUSIONS: This study shows that serum s-IL-2-R level at the time of diagnosis represents a new independent prognostic variable for predicting the risk of locoregional recurrence and survival for patients with head and neck squamous cell carcinoma.

16/7/2 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09010730 97038638
Activation of adenosine A3 receptors on macrophages inhibits tumor necrosis factor-alpha.

McWhinney CD; Dudley MW; Bowlin TL; Peet NP; Schook L; Bradshaw M; De M; Borcherding DR; Edwards CK 3rd

Department of Immunology, Marion Merrell Dow Research Institute, Marion Merrell Dow Pharmaceuticals, Inc., Cincinnati, OH 45215-6300, USA.

Eur J Pharmacol (NETHERLANDS) Aug 29 1996, 310 (2-3) p209-16, ISSN 0014-2999 Journal Code: EN6

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Murine macrophage-derived tumor necrosis factor alpha (TNF-alpha) gene expression has been shown to be dramatically induced by bacterial lipopolysaccharide, and to be dependent upon nuclear factor-kappa B (NF-kappa B) binding sites in its promoter for the lipopolysaccharide induction. Murine J774.1 macrophage cells were found to predominantly express the adenosine A3 receptor RNA relative to adenosine A1 receptor or adenosine A2 receptor RNA. Adenosine receptor agonists, in a dose-dependent manner characteristic of the adenosine A3 receptor, blocked the endotoxin induction of the TNF-alpha gene and TNF-alpha protein expression in the J774.1 macrophage cell line. The adenosine A3 receptor antagonist BW-1433 dose-dependently reversed this adenosine *inhibitory* effect on *TNF*-alpha gene expression. Thus, the binding of adenosine receptor agonists to the adenosine A3 receptor interrupts the endotoxin CD14 receptor signal transduction pathway and blocks induction of cytokine TNF-alpha, revealing a novel cross-talk between the murine adenosine A3 receptor and the endotoxin CD14 receptor in J774.1 macrophages.

16/7/3 (Item 3 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

09000017 97086967
Mouse islet cell lysis mediated by interleukin-1-induced Fas.
Yamada K; Takane-Gyotoku N; Yuan X; Ichikawa F; Inada C; Nonaka K
Department of Medicine, Kurume University School of Medicine, Japan.
Diabetologia (GERMANY) Nov 1996, 39 (11) p1306-12, ISSN 0012-186X
Journal Code: E93
Languages: ENGLISH
Document type: JOURNAL ARTICLE
This study was conducted to investigate the possible involvement of Fas in beta-cell death in insulitis of Type 1 (insulin-dependent) diabetes mellitus. Although primary cultured Balb/c mouse islet cells did not express Fas mRNA, 4-12 hours of treatment with 10(2)-10(3) U/l of mouse interleukin-1 alpha (IL-1 alpha) induced the expression of Fas mRNA. Surface Fas expression was detected by immunofluorescence flow cytometry using a non-cytolytic anti-Fas monoclonal antibody after 6 or 12 h of incubation with 10(3) U/l of IL-1 alpha. Primary islet cells were resistant to an agonistic anti-Fas monoclonal antibody. However, 12 h pretreatment with IL-1 alpha sensitized islet cells to its cytolytic effect. Significant cell death was observed 24 h after the addition of anti-Fas, and progressively increased until 72 h, when specific 51Cr release was 72 +/- 6%. Agarose gel electrophoresis of DNA extracted from cells exposed to IL-1 alpha and agonistic anti-Fas showed internucleosomal DNA fragmentation, a hallmark of apoptotic cell death. Since the Fas antibody showed no cross-reactive activity of tumour necrosis factor (TNF), the cytotoxic effect was not mediated by *TNF* receptors. A protein synthesis *inhibitor* cycloheximide augmented Fas-mediated islet cell death. The Fas-mediated killing of islet cells was not L-arginine-dependent, or blocked by N(G)-monomethyl-L-arginine. beta-TC1 cells also expressed Fas mRNA when exposed to IL-1 alpha or IL-1 alpha plus interferon-gamma. These observations suggest that Fas-mediated apoptosis may be a mechanism of islet cell death in autoimmune insulitis.

16/7/4 (Item 4 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08871345 97116849
Expression of TNF and the 55-kDa TNF receptor in epidermis, oral mucosa, lichen planus and squamous cell carcinoma.
Younes F; Quartey EL; Kiguwa S; Partridge M
Epithelial Cell Biology Unit, King's College School of Medicine and Dentistry, London, UK.
Oral Dis (ENGLAND) Mar 1996, 2 (1) p25-31, ISSN 1354-523X
Journal Code: CGI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
TNF has diverse biological effects including a role in the immune response and growth regulatory effects. OBJECTIVES: The aim of this study was to determine the nature and location of cells able to synthesise and respond to TNF in oral mucosa in health and disease. MATERIALS AND METHODS: The location of immunoreactive TNF and 55-kDa and 75-kDa TNF receptors was demonstrated using immunohistology. We also used RT-PCR to help determine the sites of synthesis of this cytokine in oral mucosa. RESULTS: Only occasional TNF-positive cells were detected in normal epidermis and oral mucosa. However, this cytokine was found throughout the epithelia in oral lichen planus, leukoplakia and squamous cell carcinoma (SCC). The possible cellular sources and biological effects of TNF in these disease processes is discussed. The 55-kDa TNFR was expressed at the cell membrane throughout the epidermis, but confined to the lower cell layers on oral mucosa. Ten of

22 SCC did not *express* this receptor. A significant correlation was observed between the velocity of tumour growth and absence of *TNF* receptors. CONCLUSIONS: The growth *inhibitory* and immunoregulatory effects of *TNF* may be modulated by changes in receptor expression and alterations in synthesis of this cytokine in a subgroup of oral SCC.

16/7/5 (Item 5 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08795391 97012163
Endogenous *tumor* *necrosis* *factor* enhances topoisomerase II *inhibitors* activity in human ovarian cancer cell lines.
Debernardis D; Stanzione S; Ottoboni C; Clerico L; Mancuso T; Parodi S; Russo P
Department of Experimental Oncology, Istituto Nazionale per la Ricerca sul Cancro, Genova-Italia.
J Pharmacol Exp Ther (UNITED STATES) Oct 1996, 279 (1) p84-90, ISSN 0022-3565 Journal Code: JP3
Languages: ENGLISH
Document type: JOURNAL ARTICLE
In this study, we demonstrated that tumor necrosis factor (TNF), secreted endogenously by four human ovarian cancer cell lines (A2774, IGROV-1, OVCAR-8, SW626), is biologically active against L929 cells and its activity is specifically inhibited by anti-TNF antibodies. Its endogenous production is increased by treatment for 24 h with phorbol myristate acetate (PMA)/ Ionomycin (Iono). All cell lines express *TNF* high-affinity *receptors* and release only 60-kdalton *soluble* *TNF* *receptor*, both spontaneously and after stimulation with PMA/Iono. TNF endogenously secreted by human ovarian cancer cell lines is very efficient in potentiating the activity of DNA topoisomerase II inhibitors (doxorubicin, mitoxantrone, VP16). The activity of vinblastine and bleomycin is not potentiated and, more interestingly, cisplatin's activity is inhibited. In 24-h PMA/Iono-stimulated A2774 cells, mitoxantrone specifically generated more cleavable complexes than in unstimulated cells. This result could provide an important tool in the therapy of human ovarian cancer secreting TNF protein, previously considered as a negative prognostic factor.

16/7/6 (Item 6 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08792217 96430819
Soluble *tumor* *necrosis* *factor* *receptor* inhibits interleukin 12 production by stimulated human adult microglial cells in vitro.
Becher B; Dodelet V; Fedorowicz V; Antel JP
Montreal Neurological Institute, Department of Neurology and Neurosurgery, McGill University, Canada.
J Clin Invest (UNITED STATES) Oct 1 1996, 98 (7) p1539-43, ISSN 0021-9738 Journal Code: HS7
Languages: ENGLISH
Document type: JOURNAL ARTICLE
IL-12 is a cytokine detected in active lesions in multiple sclerosis (MS) and promotes the acquisition of a Th1 cytokine profile by CD4+ T cells. Autoreactive T cells recovered from the central nervous system of animals with experimental autoimmune encephalomyelitis (EAE), a disease model for MS, display this phenotype. We demonstrate that human central nervous system-derived microglia, but not astroglia, can produce IL-12 in vitro.

Under basal culture conditions, human adult microglia do not express detectable levels of IL-12, although these cells show some degree of activation as assessed by expression of the immunoregulatory surface molecules HLA-DR and B7 as well as low levels of TNF-alpha mRNA. Following activation with LPS, IL-12 p40 mRNA and p70 protein can be readily detected. IL-12 production is preceded by TNF-alpha production and is inhibited by recombinant *soluble* human *TNF* *receptor* (II)-IgG1 fusion protein (shu-*TNF* -R). These data indicate regulation of IL-12 by an autocrine-dependent feedback loop, providing an additional mechanism whereby shu-TNF-R, now used in clinical trials in MS, may be exerting its effect.

16/7/7 (Item 7 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08776382 96344069
Interferon-gamma inhibits the synthesis and release of renin from human decidual cells.
Jikihara H; Poisner AM; Handwerger S
Division of Endocrinology, Children's Hospital Medical Center and Perinatal Research Institute, University of Cincinnati College of Medicine, Ohio 45229, USA.
Biol Reprod (UNITED STATES) Jun 1996, 54 (6) p1311-6, ISSN 0006-3363 Journal Code: A3W
Contract/Grant No.: HD-15201, HD, NICHD
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Experiments were performed to examine the effect of interferon-gamma (IFN gamma) on the expression of renin by human uterine decidual cells and decidual macrophages. Exposure of a mixed population of decidual cells consisting of 80% decidualized stromal cells and 20% macrophages to IFN gamma for 4 days caused a dose-dependent inhibition of renin release beginning 2 days after exposure. Renin release on Day 4 was inhibited by a maximum of 83.9%, and the half-maximal effective dose of IFN gamma was 5 ng/ml (290 pM). The inhibition of renin release in response to IFN gamma was accompanied by a comparable inhibition of renin mRNA levels. In addition to inhibiting basal renin expression, IFN gamma potentiated the *inhibitory* effect of *tumor* *necrosis* *factor* alpha (TNF alpha) on renin expression. IFN gamma also inhibited basal renin release and potentiated the *inhibitory* effect of *TNF* alpha by highly purified populations of decidual stromal cells and decidual macrophages prepared by immunomagnetic separation with beads coupled to an anti-human leukocyte antigen (HLA-DR) antibody that binds macrophages but not stromal cells. Reverse transcription-polymerase chain reaction analysis showed that HLA-DR(+) cells *express* IFN gamma mRNA, and that both HLA-DR(+) and HLA-DR(-) cells *express* IFN gamma receptors. Since IFN gamma is expressed only by decidual macrophages, the results of this study strongly suggest that IFN gamma inhibits the expression of decidual renin by a paracrine action.

16/7/8 (Item 8 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08765097 96165397
IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance.

Hotamisligil GS; Peraldi P; Budavari A; Ellis R; White MF; Spiegelman BM
Department of Cellular and Molecular Biology, Dana-Farber Cancer Institute, Boston, MA, USA.
Science (UNITED STATES) Feb 2 1996, 271 (5249) p665-8, ISSN 0036-8075 Journal Code: UJ7
Contract/Grant No.: DK 42539, DK, NIDDK
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Tumor necrosis factor-alpha (TNF-alpha) is an important mediator of insulin resistance in obesity and diabetes through its ability to decrease the tyrosine kinase activity of the insulin receptor (IR). Treatment of cultured murine adipocytes with TNF-alpha was shown to induce serine phosphorylation of insulin receptor substrate 1 (IRS-1) and convert IRS-1 into an inhibitor of the IR tyrosine kinase activity in vitro. Myeloid 32D cells, which lack endogenous IRS-1, were resistant to TNF-alpha-mediated inhibition of IR signaling, whereas transfected 32D cells that *express* IRS-1 were very sensitive to this effect of *TNF*-alpha. An *inhibitory* form of IRS-1 was observed in muscle and fat tissues from obese rats. These results indicate that TNF-alpha induces insulin resistance through an unexpected action of IRS-1 to attenuate insulin receptor signaling.

16/7/9 (Item 9 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08659911 96252270
Proinflammatory agents, IL-8 and IL-10, upregulate inducible nitric oxide synthase expression and nitric oxide production in avian osteoclast-like cells.
Sunyer T; Rothe L; Jiang X; Osdoby P; Collin-Osdoby P
Department of Biology, Washington University, St. Louis, Missouri 63130, USA.
J Cell Biochem (UNITED STATES) Mar 15 1996, 60 (4) p469-83, ISSN 0730-2312 Journal Code: HNF
Contract/Grant No.: AR32927, AR, NIAMS; DE11060, DE, NIDR
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Nitric oxide synthase (NOS) isoenzymes generate nitric oxide (NO), a sensitive multifunctional intercellular signal molecule. High NO levels are produced by an inducible NOS (iNOS) in activated macrophages in response to proinflammatory agents, many of which also regulate local bone metabolism. NO is a potent inhibitor of osteoclast bone resorption, whereas inhibitors of NOS promote bone resorption both in vitro and in vivo. The possibility that osteoclasts, like macrophages, *express* a regulated iNOS and produce NO as a potential autocrine signal following inflammatory stimulation was investigated in well-characterized avian marrow-derived osteoclast-like cells. NO production (reflected by medium nitrite levels) was markedly elevated in these cells by the proinflammatory agents lipopolysaccharide (LPS) and the synergistic action of IL-1 alpha, *TNF* alpha, and IFN gama. *inhibitors* of NOS activity (aminoguanidine, L-NAME) or iNOS induction (dexamethasone, TGF beta) reduced LPS-stimulated nitrite production. LPS also increased the NOS-associated diaphorase activity of these cells and their reactivity with anti-iNOS antibodies. RT-PCR cloning, using avian osteoclast-like cell RNA and human iNOS primers, yielded a novel 900 bp cDNA with high sequence homology (76%) to human, rat, and mouse iNOS genes. In probing osteoclast-like cell RNA with the PCR-derived iNOS cDNA, a 4.8 kb mRNA species was detected whose levels were greatly increased by LPS. Induction of iNOS mRNA by LPS, or by proinflammatory cytokines, occurred prior to the rise of medium nitrite in time course studies and was

diminished by dexamethasone. Moreover, osteoclast-like cells demonstrated an upregulation of NO production and iNOS mRNA by IL-8 and IL-10, regulatory mechanism's not previously described. It is concluded that osteoclast-like cells *express* a novel iNOS that is upregulated by inflammatory mediators, leading to NO production. Therefore, NO may serve as both a paracrine and autocrine signal for modulating osteoclast bone resorption.

16/7/10 (Item 10 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08601024 96231791
Tumor necrosis factor-alpha and tumor necrosis factor receptors in the failing human heart.
Torre-Amione G; Kapadia S; Lee J; Durand JB; Bies RD; Young JB; Mann DL
Department of Medicine, Veterans Administration Medical Center, Houston, TX 77030, USA.
Circulation (UNITED STATES) Feb 15 1996, 93 (4) p704-11, ISSN 0009-7322 Journal Code: DAW
Contract/Grant No.: P50-HL-06H, HL, NHLBI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
BACKGROUND: Tumor necrosis factor-alpha (TNF-alpha) is a proinflammatory cytokine that produces negative inotropic effects in the heart. Recently, elevated levels of TNF-alpha have been reported in patients with advanced congestive heart failure. Although TNF-alpha is thought to exert its deleterious effects by binding to two cell surface receptors, TNFR1 and TNFR2, the level of expression and regulation of TNF receptors in the heart in cardiac disease states is not known. METHODS AND RESULTS: We examined mRNA and protein levels for TNFR1, TNFR2, and TNF-alpha in explanted hearts from organ donors as well as in patients with end-stage dilated cardiomyopathy (DCM) and ischemic heart disease (IHD). Northern blot analysis revealed that mRNA for TNFR1 and TNFR2 was present in nonfailing, DCM, and IHD hearts. TNFR1 and TNFR2 receptor protein levels, as measured by ELISA, were decreased 60% in DCM and IHD patients compared with nonfailing hearts ($P < .005$). To determine a potential mechanism for the decrease in TNF receptor expression, we measured levels of circulating *soluble* *TNF* *receptors* (sTNFRs) in DCM and IHD patients. This analysis showed that there was a significant one-and-a-half to threefold increase in sTNFRs in DCM ($P < .03$) and IHD patients ($P < .001$). Another important finding was that TNF-alpha mRNA and TNF-alpha protein were present in the explanted hearts from DCM and IHD patients but not in nonfailing hearts. CONCLUSIONS: In summary, the results of this study constitute the initial demonstration that TNF receptor proteins are dynamically regulated in patients with advanced congestive heart failure. Moreover, the observation that failing hearts *express* elevated levels of TNF-alpha suggests that overexpression of this cytokine may be one of several different maladaptive mechanisms responsible for the progressive cardiac decompensation that occurs in advanced heart failure.

16/7/11 (Item 11 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08582791 96199244
Distinct roles of the two tumor necrosis factor (TNF) receptors in modulating TNF and lymphotoxin alpha effects.

Medvedev AE; Espevik T; Ranges G; Sundan A

Institute of Cancer Research and Molecular Biology, Trondheim, Norway.

J Biol Chem (UNITED STATES) Apr 19 1996, 271 (16) p9778-84, ISSN 0021-9258 Journal Code: HIV

Languages: ENGLISH

Document type: JOURNAL ARTICLE

The role for the two tumor necrosis factor (TNF) receptors in discriminating TNF and lymphotoxin alpha (LTalpha) effects has been studied. TNF and LTalpha were equally mitogenic in Fs4 fibroblasts, which *express* a high amount of the p55 compared to the p75 TNF receptors (TNFRs). In contrast, TNF was more potent than LTalpha in mediating gene regulation and cytotoxicity in SW480-betaGal cells and KYM-1 cells, which have a high p75/p55 TNFR ratio. Both TNF and LTalpha showed comparable affinities for the two TNFRs. However, in contrast to LTalpha, TNF dissociated rapidly from the p75 TNFR, whereas both cytokines dissociated slowly from the p55 TNFR. Soluble p55 TNFR was much more potent than soluble p75 TNFR in inhibiting *TNF* cytotoxicity, whereas both *soluble* *receptors* moderately decreased LTalpha-mediated cytotoxicity with comparable efficacy. Antagonistic monoclonal antibodies against either TNFR types markedly inhibited TNF effects. However, only the p55 TNFR antagonistic antibody significantly decreased LTalpha-mediated cytotoxicity and cytomegalovirus promoter activation, whereas blocking of the p75 TNFR enhanced the LTalpha effects. These data suggest that whereas the p75 TNFR can both directly propagate TNF signals and "pass" TNF to the p55 TNFR, it attenuates LTalpha and may serve as a decoy receptor for this cytokine.

16/7/12 (Item 12 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

08501961 96121002

Gastrin secretion from primary cultures of rabbit antral G cells: stimulation by inflammatory cytokines.

Weigert N; Schaffer K; Schusdziarra V; Classen M; Schepp W

Second Department of Internal Medicine, Technical University of Munich, Germany.

Gastroenterology (UNITED STATES) Jan 1996, 110 (1) p147-54, ISSN 0016-5085 Journal Code: FH3

Languages: ENGLISH

Document type: JOURNAL ARTICLE

BACKGROUND & AIMS: In Helicobacter pylori-induced gastritis, local production of cytokines may favor hypergastrinemia as an endocrine link between H. pylori-induced gastritis and duodenal ulcer. The aim of this study was to characterize cytokine effects on cultured rabbit antral G cells. METHODS: Monolayers (14.2% +/- 2.9% G cells) were studied after 48 hours in primary culture. RESULTS: Interleukin (IL) 1 beta (50% effective concentration [EC50], 5.3 +/- 0.4 ng/mL) and tumor necrosis factor (TNF) alpha (EC50, 5.5 +/- 0.5 ng/mL) stimulated gastrin release to 50% of the maximal response to 10(-9) mol/L neuromedin C. Stimulation by the maximally effective concentration of IL-1 beta (10 ng/mL) was inhibited by the human IL-1 receptor antagonist (100 ng/mL; inhibitory constant, 23.0 ng/mL), which prefers type I over type II IL-1 receptors. The response to the maximally effective concentration of TNF-alpha (10 ng/mL) was markedly inhibited by monoclonal antibody H398, an antagonist at *TNF* P55 receptors (*inhibitory* constant, 1.7 micrograms/mL), whereas monoclonal antibody utr1, an antagonist at TNF P75 receptors, was ineffective. Stimulation by IL-1 beta and TNF-alpha was additive to the responses to neuromedin C and O2-dibutyryl adenosine 3',5'-cyclic monophosphate. IL-6 and IL-8 (0.1-50 ng/mL) were ineffective. CONCLUSIONS: IL-1 beta and TNF-alpha stimulate

gastrin secretion via receptors potentially residing on rabbit antral G cells themselves. We speculate that G cells *express* type I IL-1 receptors and TNF P55 but not TNF P75 receptors.

16/7/13 (Item 13 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08494316 96098555
Tumor-associated lymphocytes (TAL) are competent to produce higher levels of cytokines in neoplastic pleural and peritoneal effusions than those found in sera and are able to release into culture higher levels of IL-2 and IL-6 than those released by PBMC.

Mantovani G; Maccio A; Versace R; Pisano M; Lai P; Esu S; Ghiani M; Dessi D; Turnu E; Santona MC; et al
Department of Medical Oncology, University of Cagliari, Italy.

J Mol Med (GERMANY) Aug 1995, 73 (8) p409-16, ISSN 0946-2716
Journal Code: B8C
Languages: ENGLISH
Document type: JOURNAL ARTICLE

This work was designed to study the proliferative response of tumor-associated lymphocytes (TAL) from neoplastic effusions against autologous tumor cells and the immunophenotype pattern of TAL from neoplastic effusions and that of PBMC of the same patients. We also compared the serum levels of the cytokines interleukin (IL) 1 beta, 2 and 6, *tumor* *necrosis* *factor*-alpha (*TNF* alpha) and *soluble* IL-2 *receptor* (sIL-2R) with those present in neoplastic effusions of the same patients. Moreover, we examined the ability of TAL and peripheral blood mononuclear cells (PBMC) to produce and release the cytokines and sIL-2R and to *express* membrane CD25 following their stimulation with phytohemagglutinin (PHA) in vitro. Finally, we compared the cytokines/sIL-2R production and membrane CD25 expression by PHA-stimulated PBMC of the patients with neoplastic effusions with a series of 90 cancer patients without neoplastic effusions and 20 normal healthy subjects. Thirteen neoplastic pleural and eight peritoneal effusions were collected from 11 patients with primary lung cancer, 7 with primary epithelial ovarian cancer, 1 with breast cancer, 1 with pleural mesothelioma, and 1 with pancreatic cancer. The proliferative response of TAL from neoplastic effusions against autologous tumor cells was lower than the response to PHA, IL-2, and anti-CD3, but significant. The percentage distribution of CD3+ and CD8+ lymphocyte subpopulations was higher in peritoneal than in pleural effusions, while the CD16+ subset was higher in pleural than in peritoneal effusions. The percentage distribution of CD16+ was significantly lower in pleural effusions than in PBMC of patients with pleural effusions.(ABSTRACT TRUNCATED AT 250 WORDS)

16/7/14 (Item 14 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08438837 96070657
Increased expression of Fas antigen on bone marrow CD34+ cells of patients with aplastic anaemia.

Maciejewski JP; Selleri C; Sato T; Anderson S; Young NS
Hematology Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892-1652, USA.

Br J Haematol (ENGLAND) Sep 1995, 91 (1) p245-52, ISSN 0007-1048
Journal Code: AXC

Languages: ENGLISH
Document type: JOURNAL ARTICLE

Fas antigen, a receptor molecule that mediates signals for programmed cell death, is involved in T-cell-mediated killing of malignant, virus-infected or allogeneic target cells. Interferon-gamma (IFN-gamma) and *tumour* *necrosis* *factor*-alpha (*TNF*-alpha), potent *inhibitors* of haemopoiesis, enhance Fas receptor expression on bone marrow (BM) CD34+ cells, and both cytokines render haemopoietic progenitor cells susceptible to Fas-mediated inhibition of colony formation due to the induction of apoptosis. Haemopoietic suppression in aplastic anaemia (AA) has been associated with aberrant IFN-gamma, increased TNF-beta expression, and elevated numbers of activated cytotoxic T-cells in marrow. We have now examined Fas antigen expression in fresh AA BM samples. In normal individuals few CD34+ cells expressed Fas antigen and normal marrow cells had low sensitivity to Fas-mediated inhibition of colony formation. In contrast, in early AA, BM CD34+ cells showed markedly increased percentages of Fas receptor-expressing CD34+ cells, which correlated with increased sensitivity of AA marrow cells to anti-Fas antibody-mediated inhibition of colony formation. The proportion of Fas antigen-bearing cells was lower in recovered patients' BM. Fas antigen was also detected in the marrow of some patients with myelodysplasia, especially the hypocellular variant. These results are consistent with the hypothesis that AA CD34+ cells, probably including haemopoietic progenitor cells, *express* high levels of Fas receptor due to in vivo exposure to IFN-gamma and/or TNF-alpha and are suitable targets for T-cell-mediated killing. Our results suggest that the Fas receptor/Fas ligand system are involved in the pathophysiology of BM failure.

16/7/15 (Item 15 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08432481 96032951

In vitro stimulation of human endothelial cells by sera from a subpopulation of high-percentage panel-reactive antibody patients.

Pidwell DJ; Heller MJ; Gabler D; Orosz CG

Department of Surgery, Ohio State University College of Medicine, Columbus 43210, USA.

Transplantation (UNITED STATES) Sep 27 1995, 60 (6) p563-9, ISSN 0041-1337 Journal Code: WEJ

Contract/Grant No.: RO1 AI33589, AI, NIAID; P30-CA1605814, CA, NCI

Languages: ENGLISH

Document type: JOURNAL ARTICLE

We have studied a serum activity that enhances in vitro ICAM-1 expression by human endothelial cells (EC) and report that this activity can be found in approximately 8% of pretransplant serum samples from individuals with a history of high %PRA. Hence, most high %PRA sera lack this activity, and, furthermore, mixing these negative sera does not result in an active serum pool. In patients with active serum, the ICAM-1 enhancing activity is found only sporadically, despite the continuous detection of endothelial-reactive antibodies. Absorption of Ig from a high %PRA serum reduced ICAM-1 enhancing activity, as well as endothelial-reactive antibodies. However, enhancing activity can sometimes be observed in sera that lack detectable endothelial-reactive antibodies, and none of several patient sera with defined MHC class I-specific alloantibodies displayed ICAM-1 enhancing activity. Together, these data suggest that ICAM-1 enhancing activity may not necessarily be mediated by anti-MHC alloantibodies. In addition to influencing this expression, ICAM-1 active patient sera also influence EC expression of VCAM-1 and MHC class I, but not MHC class II molecules, a

pattern that is similar to that stimulated by TNF alpha. However, coincubation of EC with active serum plus *soluble* *TNF* *receptor* did not block the endothelial phenotypic changes, despite the ability of the soluble receptor to completely abrogate endothelial changes induced by TNF alpha. IFN gamma also increases endothelial ICAM-1 expression, but has response kinetics different from that of active serum. Interestingly, brief treatment of endothelial cells with IFN gamma greatly increased the amount of IgG bound from the active sera by EC. We conclude that some pretransplant patients occasionally *express* an activity in their serum that influences EC expression of several adhesion molecules, including ICAM-1, VCAM-1, and MHC class I. This activity may be associated with alloantibodies, but is independent of MHC class I-reactive antibodies, circulating TNF alpha, or IFN gamma. The relevance of a serum-borne component capable of activating EC is discussed.

16/7/16 (Item 16 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08402611 95276251
Fas antigen expression on CD34+ human marrow cells is induced by interferon gamma and tumor necrosis factor alpha and potentiates cytokine-mediated hematopoietic suppression in vitro.
Maciejewski J; Selleri C; Anderson S; Young NS
Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA.
Blood (UNITED STATES) Jun 1 1995, 85 (11) p3183-90, ISSN 0006-4971
Journal Code: A8G
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Activation of Fas antigen, a cell surface receptor molecule, by its ligand results in transduction of a signal for cell death. The Fas system has been implicated in target cell recognition, clonal development of immune effector cells, and termination of the cellular immune response. Fas antigen expression on lymphocytes is regulated by interferon gamma (IFN gamma) and tumor necrosis factor alpha (*TNF* alpha), cytokines that also have *inhibitory* effects on hematopoiesis. We investigated Fas antigen expression on human marrow cells and the effects of Fas activation on hematopoiesis in vitro. Freshly isolated immature hematopoietic cells, as defined by the CD34 marker, did not *express* Fas antigen at levels detectable by fluorescent staining. CD34+ cells, which include progenitors and stem cells, showed low levels of Fas expression in culture, even in the presence of growth factors. Stimulation by TNF alpha and IFN gamma markedly increased Fas antigen expression on CD34+ cells. Anti-Fas antibody, which mimics the action of the putative ligand, enhanced IFN gamma- and TNF alpha-mediated suppression of colony formation by bone marrow (BM) in a dose-dependent manner. This effect did not require the presence of accessory cells. Colony formation from mature (CD34+ CD38+) and immature (CD34+ CD38-) progenitor cells and long-term culture initiating cells were susceptible to the inhibitory action of anti-Fas antibody in the presence of IFN gamma and TNF alpha. Apoptosis assays performed on total BM cells and CD34+ cells showed that anti-Fas antibody induced programmed cell death of CD34+ BM cells.(ABSTRACT TRUNCATED AT 250 WORDS)

16/7/17 (Item 17 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08395395 95138688
Intraislet release of interleukin 1 inhibits beta cell function by inducing beta cell expression of inducible nitric oxide synthase.
Corbett JA; McDaniel ML
Department of Pathology, Washington University School of Medicine, St. Louis, Missouri 63110.
J Exp Med (UNITED STATES) Feb 1 1995, 181 (2) p559-68, ISSN 0022-1007 Journal Code: I2V
Contract/Grant No.: DK-06181, DK, NIDDK; F32 DK-08748, DK, NIDDK
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Cytokines, released in and around pancreatic islets during insulitis, have been proposed to participate in beta-cell destruction associated with autoimmune diabetes. In this study we have evaluated the hypothesis that local release of the cytokine interleukin 1 (IL-1) by nonendocrine cells of the islet induce the expression of inducible nitric oxide synthase (iNOS) by beta cells which results in the inhibition of beta cell function. Treatment of rat islets with a combination of tumor necrosis factor (TNF) and lipopolysaccharide (LPS), conditions known to activate macrophages, stimulate the expression of iNOS and the formation of nitrite. Although TNF+LPS induce iNOS expression and inhibit insulin secretion by intact islets, this combination does not induce the expression of iNOS by beta or alpha cells purified by fluorescence activated cell sorting (Facs). In contrast, IL-1 beta induces the expression of iNOS and also inhibits insulin secretion by both intact islets and Facs-purified beta cells, whereas *TNF*+LPS have no *inhibitory* effects on insulin secretion by purified beta cells. Evidence suggests that TNF+LPS inhibit insulin secretion from islets by stimulating the release of IL-1 which subsequently induces the expression of iNOS by beta cells. The IL-1 receptor antagonist protein completely prevents TNF+LPS-induced inhibition of insulin secretion and attenuates nitrite formation from islets, and neutralization of IL-1 with antisera specific for IL-1 alpha and IL-1 beta attenuates TNF+LPS-induced nitrite formation by islets. Immunohistochemical localization of iNOS and insulin confirm that TNF+LPS induce the expression of iNOS by islet beta cells, and that a small percentage of noninsulin-containing cells also *express* iNOS. Local release of IL-1 within islets appears to be required for TNF+LPS-induced inhibition of insulin secretion because TNF+LPS do not stimulate nitrite formation from islets physically separated into individual cells. These findings provide the first evidence that a limited number of nonendocrine cells can release sufficient quantities of IL-1 in islets to induce iNOS expression and inhibit the function of the beta cell, which is selectively destroyed during the development of autoimmune diabetes.

16/7/18 (Item 18 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08390987 95052653
Polyclonal B cell activation induced by herpesvirus saimiri-transformed human CD4+ T cell clones. Role for membrane TNF-alpha/TNF-alpha receptors and CD2/CD58 interactions.
Del Prete G; De Carli M; D'Elios MM; Fleckenstein IM; Fickenscher H; Fleckenstein B; Almerigogna F; Romagnani S
Department of Allergology and Clinical Immunology, University of Florence, Italy.
J Immunol (UNITED STATES) Dec 1 1994, 153 (11) p4872-9, ISSN 0022-1767 Journal Code: IFB
Languages: ENGLISH

Document type: JOURNAL ARTICLE

We have shown that in vitro infection herpesvirus saimiri (HVS) can transform human CD4+ T cell clones with defined Th1 or Th2 cytokine profiles to continuous growth. We report here that transformation with HVS enabled both Th1 and Th2 clones to stimulate proliferation and Ig production by autologous or allogeneic B cells in the absence of stimulants. The polyclonal B cell-activating property of HVS-transformed clones was not related to free virus or soluble cytokines, but rather was dependent on an Ag-nonspecific, MHC-unrestricted, contact-dependent mechanism. T blasts from unstimulated HVS-transformed clones did not *express* CD40 ligand (CD40L) mRNA or CD40L protein, whereas a proportion of them constitutively expressed membrane TNF (mTNF)-alpha. Both CD40L and mTNF-alpha were detectable on either uninfected or HVS-transformed clones upon mitogen stimulation. The activation of high-density B cells by unstimulated HVS-transformed clones was not inhibited by soluble CD40-Ig fusion protein, but was strongly reduced by either anti-TNF-alpha or anti-TNF-alpha receptor (TNF-alpha R) mAbs. Addition of anti-CD2 and/or anti-CD58 mAbs was also inhibitory, but no additive effect with anti-TNF-alpha and/or anti-TNF-alpha R mAbs was observed. Neither anti-IL-2 nor CD40-Ig inhibited the proliferation of naive IgD+ B cells cocultured with fixed unstimulated HVS-transformed clones, whereas a combination of anti-TNF-alpha and anti-*TNF*-alpha R mAbs was *inhibitory*. In addition, fixed unstimulated HVS-transformed clones induced Ig synthesis in IgD+ naive B cells even in the absence of exogenous IL-2. Data suggest that both the mTNF-alpha/TNF-alpha R and the CD2/CD58 pathways, but not the CD40L-CD40 interaction plus secreted cytokines, are involved in the unusual mode of B cell activation exerted by CD4+ HVS-transformed clones.

16/7/19 (Item 19 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08382732 94267470

Induction and regulation of nitric oxide synthase in retinal Muller glial cells.

Goureau O; Hicks D; Courtois Y; De Kozak Y

INSERM, U 118, Unite de Recherches Gerontologiques, Association Claude Bernard, Paris, France.

J Neurochem (UNITED STATES) Jul 1994, 63 (1) p310-7, ISSN 0022-3042 Journal Code: JAV

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Muller glial cells from the rat retina were examined for their capacity to produce nitric oxide (NO). Treatment of retinal Muller glial (RMG) cells with lipopolysaccharide (LPS), interferon-gamma, and tumor necrosis factor-alpha induced NO synthesis as determined by nitrite release in media. Simultaneous addition of LPS, interferon-gamma, and tumor necrosis factor-alpha caused the largest increase in NO synthesis. NO biosynthesis was detected after 12 h and was dependent on the dose of LPS, interferon-gamma, and *tumor* *necrosis* *factor*-alpha. Stereoselective *inhibitors* of NO synthase (NOS), cycloheximide and transforming growth factor-beta, blocked cytokine-induced NO production. Cytosol from LPS/cytokine-treated RMG cultures, but not from unstimulated cultures, produced a calcium/calmodulin-independent conversion of L-arginine to L-citrulline that was completely blocked by NOS inhibitor. The expression of NOS in RMG cells was confirmed by northern blot analysis, in which stimulation of these cells led to an increase in NOS mRNA levels. We conclude that RMG cells can *express* an inducible form of NOS similar to the macrophage isoform. High NO release from activated RMG cells might

represent a protection from infection but may also contribute to the development of retinal pathologies.

16/7/20 (Item 20 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08377184 94125031
NF-kappa B and I kappa B alpha: an inducible regulatory system in endothelial activation.
Read MA; Whitley MZ; Williams AJ; Collins T
Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts.
J Exp Med (UNITED STATES) Feb 1 1994, 179 (2) p503-12, ISSN 0022-1007 Journal Code: I2V
Contract/Grant No.: HL-45462, HL, NHLBI; HL-35716, HL, NHLBI; PO1 36028; +
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Structural analysis of the promoters of several endothelial genes induced at sites of inflammatory or immune responses reveals binding sites for the transcription factor nuclear factor kappa B (NF-kappa B). Endothelial cells *express* transcripts encoding the p50/p105 and p65 components of NF-kappa B and the rel-related proto-oncogene c-rel; steady state levels of these transcripts are transiently increased by tumor necrosis factor alpha (TNF-alpha). Western blotting revealed that stimulation of endothelial cells with TNF-alpha resulted in nuclear accumulation of the p50 and p65 components of NF-kappa B. Ultraviolet crosslinking and immunoprecipitation demonstrated binding of the p50 and p65 components of NF-kappa B to the E-selectin kappa B site. Endothelial cells *express* an inhibitor of NF-kappa B activation, I kappa B-alpha (MAD-3). Protein levels of this *inhibitor* fall rapidly after *TNF*-alpha stimulation. In parallel, p50 and p65 accumulate in the nucleus and RNA transcript levels for I kappa B-alpha are dramatically upregulated. Recombinant p65 stimulates expression of E-selectin promoter-reporter constructs. I kappa B-alpha inhibits p65 or TNF-alpha-stimulated E-selectin promoter-reporter gene expression in transfected endothelial cells. The NF-kappa B and I kappa B-alpha system may be an inducible regulatory mechanism in endothelial activation.

16/7/21 (Item 21 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08272219 95287657
B-lineage lymphoid blast crisis in juvenile chronic myelogenous leukemia: II. Interleukin-1-mediated autocrine growth regulation of the lymphoblasts.
Attias D; Grunberger T; Vanek W; Estrov Z; Cohen A; Lau R; Freedman MH
Division of Hematology/Oncology, Hospital for Sick Children, Toronto, Canada.
Leukemia (ENGLAND) May 1995, 9 (5) p884-8, ISSN 0887-6924 Journal Code: LEU
Languages: ENGLISH
Document type: JOURNAL ARTICLE
A pre-B acute lymphoblastic leukemia (ALL) cell line with monosomy 7 was established from a child with juvenile chronic myelogenous leukemia (JCML) in lymphoid blast crisis. Analysis of the growth properties of the cell line, termed 'W1' showed an interleukin-1 (IL-1) mediated autocrine pattern of cell proliferation with the following features: W1 colony growth without

added growth factor was density-dependent and colony growth was augmented with serum-free autologous cell culture supernatant; exogenous IL-1 beta had a growth-promoting effect on W1 colony numbers when cells were seeded at low density; W1 cells constitutively expressed mRNA for IL-1 beta, and high levels of IL-1 beta were measured in W1 cell lysates; anti-IL-1 beta antibodies as well as IL-1 receptor antagonist markedly suppressed W1 colony growth when either was added to cultures of cells seeded without growth factors at low density; anti-GM-CSF antibodies and anti-IL-3 antibodies had no inhibitory effect on W1 colony growth. Whereas W1 colony growth was also augmented by adding IL-3, IL-4, IL-6, IL-7, GM-CSF, Steel factor and erythropoietin individually to the cultures, W1 cells did not constitutively *express* mRNA for any of these cytokines. W1 colony growth was markedly suppressed by exogenous TNF-alpha which contrasts sharply with the autocrine growth promoting effect of TNF-alpha on myelomonocytic elements of JCML in 'chronic' phase. The *inhibitory* effect of *TNF*-alpha on W1 cells was not due to downregulation of IL-1 production. The IL-1-dependent growth of W1 cells appeared to be unique because none of five other pre-B lineage ALL cell lines established as controls showed an autocrine growth loop via IL-1. W1 cells provide a valuable opportunity to examine the relationship of monosomy 7, B-lineage acute lymphoblastic leukemia, aberrant genetic expression of cytokines and their receptors, and IL-1 mediated autocrine cell growth in cancer.

16/7/22 (Item 22 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08255752 95270254
Inhibitors of dipeptidyl peptidase IV (DP IV, CD26) specifically suppress proliferation and modulate cytokine production of strongly CD26 expressing U937 cells.

Reinhold D; Bank U; Buhling F; Kahne T; Kunt D; Faust J; Neubert K; Ansorge S
Department of Internal Medicine, Otto-von-Guericke University, Magdeburg, Germany.

Immunobiology (GERMANY) Dec 1994, 192 (1-2) p121-36, ISSN 0171-2985 Journal Code: GH3
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Various studies from different laboratories have shown that the membrane ectoenzyme dipeptidyl peptidase IV (DP IV, CD26) expressed in T and NK cells is involved in the regulation of DNA synthesis and cytokine production. In this paper, we performed a biochemical and functional characterization of dipeptidyl peptidase IV on the human histiocytic lymphoma cell line U937. Using U937 clones expressing low to high levels of membrane localized CD26, we found that the synthetic reversible inhibitors of DP IV, Lys-[Z(NO2)]-thiazolidide and Lys-[Z(NO2)]-piperidide, have different effects on all functions. In U937-H cells that strongly *express* high levels of CD26, DP IV inhibitors were shown to suppress DNA synthesis and production of IL-1 beta, but stimulate the secretion of the IL-1 receptor antagonist (IL-1RA) and of *TNF*-alpha. In contrast, both *inhibitors* did not influence the cytokine production and DNA synthesis in U937-L cells exhibiting low level CD26 expression. These data support the hypothesis that CD26 plays a crucial role in proliferation and cytokine production, not only in T cells, but also in other cell systems, and that enzymatic activity is essential for its function.

16/7/23 (Item 23 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

08203369 95209276
In vitro evidence for a dual role of tumor necrosis factor-alpha in human immunodeficiency virus type 1 encephalopathy [see comments]
Wilt SG; Milward E; Zhou JM; Nagasato K; Patton H; Rusten R; Griffin DE; O'Connor M; Dubois-Dalcq M
Laboratory of Viral and Molecular Pathogenesis, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892-4160.
Ann Neurol (UNITED STATES) Mar 1995, 37 (3) p381-94, ISSN 0364-5134 Journal Code: 6AE
Contract/Grant No.: NS 26643, NS, NINDS
Comment in Ann Neurol 1995 Sep;38(3):483
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Microglial cell activation, myelin alteration, and abundant tumor necrosis factor (TNF)-alpha message have been observed in the brains of some human immunodeficiency virus type 1 (HIV-1)-infected and demented patients. We therefore used cultures of purified human microglia and oligodendrocytes derived from adult human brain to examine the role of TNF-alpha in HIV-1 encephalopathy. Human microglia synthesize TNF-alpha message and protein in vitro. When these cells were infected with HIV-1 JrFL and maintained in the presence of *TNF*-alpha antibodies, *soluble* *TNF*-alpha *receptors*, or the *TNF*-alpha *inhibitor* pentoxifylline, viral replication was delayed or strongly inhibited. Both human microglia and oligodendrocytes *express* the two TNF receptors, TNF-R1, which has been implicated in cytotoxicity, and TNF-R2. While TNF-alpha may enhance HIV-1 replication in an autocrine manner, it is not toxic for microglia. In contrast, recombinant human TNF-alpha causes oligodendrocyte death in a dose-dependent manner. In situ detection of DNA fragmentation in some cells indicated that oligodendrocyte death may occur by apoptosis. Addition of live microglia or medium conditioned by these cells also resulted in 30 to 40% oligodendrocyte death, which was largely prevented by *TNF*-alpha *inhibitors*. We propose that *TNF* -alpha plays a dual role in HIV-1 encephalopathy, enhancing viral replication by activated microglia and damaging oligodendrocytes. Thus, *TNF*-alpha *inhibitors* may alleviate some of the neurological manifestations of acquired immunodeficiency syndrome.

16/7/24 (Item 24 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08196494 95201193
TNF receptor fusion proteins are effective *inhibitors* of *TNF* -mediated cytotoxicity on human KYM-1D4 rhabdomyosarcoma cells.
Butler DM; Scallon B; Meager A; Kissonerghis M; Corcoran A; Chernajovsky Y; Feldmann M; Ghrayeb J; Brennan FM
Kennedy Institute of Rheumatology, Hammersmith, London, UK.
Cytokine (UNITED STATES) Nov 1994, 6 (6) p616-23, ISSN 1043-4666 Journal Code: A52
Languages: ENGLISH
Document type: JOURNAL ARTICLE
KYM-1D4 cells are a subline derived from a human rhabdomyosarcoma which are highly sensitive to TNF-mediated cytotoxicity. They were selected for this study because they express human TNF-R and are therefore a more relevant target for comparing the potential therapeutic value of human

TNF-*inhibitory* agents than the usual murine cell lines. Two recombinant soluble TNF-R-IgG fusion proteins, one containing p55 TNR-R, the other containing p75 TNF-R, and a recombinant monomeric soluble p55 TNF-R were all found to block the cytotoxicity generated by human TNF-alpha and LT as well as also murine TNF. The p55 TNF-R-IgG fusion protein (p55-sf2) was the most effective of the antagonists tested, requiring an equimolar, (based on a monomeric configuration of TNF-alpha) or a 3-fold higher (based on a trimeric configuration of TNF-alpha) molar concentration to inhibit the cytotoxicity mediated TNF-alpha by 50%. p55-sf2 was also as effective at inhibiting the cytotoxicity mediated by LT or murine TNF in the KYM-1D4 assay. In contrast, the monomeric soluble p55 *TNF* -R was the least effective *inhibitor* , requiring a > 4000-fold higher molar concentration than p55-sf2 to achieve a similar degree of protection. The fusion proteins, particularly p55-sf2, may be useful as human therapeutic agents, as at low concentrations they can prevent both TNF-alpha-mediated and LT-mediated effects on human cells. As TNF-R-IgG fusion proteins also block the action of murine TNF in vitro, they may also be useful in the investigation of murine models of human inflammatory disease.

16/7/25 (Item 25 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

08193019 95197154
Soluble *TNF* *receptor* production by activated T lymphocytes: differential effects of acute and chronic exposure to TNF.
Cope AP; Aderka D; Wallach D; Kahan M; Chu NR; Brennan FM; Feldmann M
Kennedy Institute of Rheumatology, Sunley Division, London, UK.
Immunology (ENGLAND) Jan 1995, 84 (1) p21-30, ISSN 0019-2805
Journal Code: GH7
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Soluble *tumour* *necrosis* *factor* *receptors* (sTNF-R) are up-regulated at sites of chronic inflammation such as rheumatoid synovial joints. The p75 sTNF-R is the more abundant, suggesting an important role for this *TNF* *inhibitor* in regulating *TNF* bioactivity in vivo. As the precise cellular source of these soluble receptors is not known, we investigated the production and regulation of sTNF-R by T lymphocytes, an abundant cell type in inflammatory infiltrates, which upon activation *express* high levels of p75 surface receptors. Using panels of T-cell lines and clones expressing high levels of p75 TNF-R, we found that p75 sTNF-R production upon stimulation is a feature common to all subsets of T cells, including cells of the CD4-CD8- double negative phenotype expressing either alpha beta or gamma delta T-cell receptors (TCR). In contrast, levels of p55 sTNF-R were only detected when T cells were stimulated at higher densities and by potent mitogens such as phorbol 12-myristate 13-acetate (PMA). Detailed kinetic analyses revealed that the production of p75 sTNF-R was biphasic, the first phase was activation dependent, occurring in the absence of detectable TNF, while the second phase of p75 sTNF-R production was regulated by cytokines such as TNF. Unlike short-term exposure to TNF which enhances sTNF-R production in vitro and in vivo, prolonged exposure of T lymphocytes to TNF suppressed p75 sTNF-R (but not p55 sTNF-R) production in a dose- and time-dependent fashion. These results suggest that in patients with chronic inflammatory disease, which are exposed to augmented levels of bioactive TNF for prolonged periods, the production of p75 sTNF-R may be impaired.

16/7/26 (Item 26 from file: 155)

DIALOG(R)File 155:MEDLINE(R)

08057609 95036350
Growth arrest and terminal differentiation of leukemic myelomonocytic cells induced through ligation of surface CD23 antigen.
Ouaaz F; Sola B; Issaly F; Kolb JP; Davi F; Mentz F; Arock M; Paul-Eugene N; Korner M; Dugas B; et al
Molecular Immuno-Hematology Group, Pitie-Salpe-triere Hospital, Paris, France.
Blood (UNITED STATES) Nov 1 1994, 84 (9) p3095-104, ISSN 0006-4971 Journal Code: A8G
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Acute myelogenous leukemia (AML) cells *express* CD23 surface antigen after in vitro treatment with various cytokines, including interleukin-4 (IL-4) and interferon gamma. Subsequent ligation of CD23 by specific monoclonal antibody (MoAb) induces substantial morphologic and functional modifications in these cells. In the present study, we investigated the role of CD23 in the proliferation and the maturation of leukemic cells from AML patients or the U937 cell line. CD23+ cell treatment with CD23 MoAb inhibited the proliferation of leukemic cells. This correlated with their terminal differentiation after 7 to 9 days incubation because they (1) definitively lost their growth capacity; (2) adhered to culture flasks and became monocyte/macrophage-like; and (3) expressed mature monocyte markers including nonspecific esterases. Intracellular mechanism of this antitumoral effect was then analyzed in U937 cells. Induction of high-density surface CD23 expression by IL-4 or granulocyte-macrophage colony-stimulating factor coincided with a transient decrease of U937 cell proliferation. CD23 ligation during this low-proliferative phase induced a rapid activation of L-arginine-dependent pathway and the intracellular accumulation of cyclic guanosine monophosphate and cyclic adenosine monophosphate (cAMP). Induction of these early messengers was followed by the activation of nuclear factor-kB transcription factor and the modulation of proto-oncogene expression by U937 cells. Whereas U937 cell treatment with IL-4 decreased c-fos/c-jun expression, CD23 MoAb reinduced c-fos/c-jun and promoted the expression of cell maturation-associated proto-oncogenes junB and c-fms, during the first 24 hours. Both IL-4 and CD23 MoAb downregulated the expression of c-myb. CD23 ligation also induced the production of *TNF* alpha by U937 cells. *Inhibitors* of cAMP and nitric oxide reversed CD23-mediated modification in U937 cells. These data evidence the ability of CD23 surface antigen to mediate terminal differentiation of early leukemic myelomonocytic cells.

16/7/27 (Item 27 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07959437 94297535
TNF receptors in chronic lymphocytic leukemia.
Waage A; Espevik T
Department of Medicine, University of Trondheim, Norway.
Leuk Lymphoma (SWITZERLAND) Mar 1994, 13 (1-2) p41-6, ISSN 1042-8194 Journal Code: BNQ
Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW; REVIEW, TUTORIAL
Two transmembrane receptors for tumor necrosis factor (TNF) with different molecular weight, 55 kD (p55) and 75 kD (p75), have been identified. In addition, the extracellular part of both receptors are

shedded by proteolytic cleavage and exist as *soluble* *receptors* which bind to *TNF* in plasma and other biological fluids. Normal B cells produce TNF and *express* TNF receptors (R), mainly p75, upon stimulation. TNF costimulates DNA synthesis via the p75 receptor in normal B cells. The B cells from patients with chronic lymphocytic leukemia (CLL) produce TNF and have the capacity to *express* both receptor types. Also in malignant B cells the p75 receptor is dominant. TNF induce DNA synthesis in CLL cells via both receptors. CLL patients have elevated serum levels of soluble (s) TNFR and this is more pronounced in advanced disease. In conclusion, there is considerable support for TNF as an autocrine growth factor in CLL. However, the net effect of TNF is determined by the quantitative relationship between TNFR on the cell surface, TNF in plasma and sTNFR in plasma, and the affinities between TNF-p55 and TNF-p75. Due to increasing serum levels of sTNFR the significance of TNF as a growth factor is probably less important in advanced disease. (34 Refs.)

16/7/28 (Item 28 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07949516 94284042
Expression and activity of MMPS and their regulators in ovarian cancer.
Naylor MS; Stamp GW; Davies BD; Balkwill FR
Biological Therapy Laboratory, Imperial Cancer Research Fund, London, UK.
Int J Cancer (UNITED STATES) Jul 1 1994, 58 (1) p50-6, ISSN 0020-7136
Journal Code: GQU
Languages: ENGLISH
Document type: JOURNAL ARTICLE
In order to understand the ability of human ovarian cancers to degrade the basement membrane, we have studied the localization and activity of matrix metalloproteases (MMPs) 2 and 9, using in situ hybridization and quantificative zymography on sequential sections of tumor biopsies. We have related these data to expression of some of the controlling elements of the enzymes, namely tissue *inhibitors* of metastasis (TIMPs) and *tumor* *necrosis* *factor* (TNF). mRNA for MMP-2 was found in the majority of cases and localized to stromal areas with maximal expression adjacent to neoplastic areas. MMP-9 expression was associated with cells in epithelial and stromal areas, consistent with distribution of macrophages. Zymography revealed higher levels of MMP-9 activity in the ovarian cancer biopsy samples than in other cancers studied, but in contrast to our previous observations in breast and bladder cancer, there was no correlation between MMP levels and tumor grade. Nor was there any association between amount of TNF mRNA and levels of MMP enzymes. TIMP-I expression was localized to stromal areas adjacent to tumor epithelial cells as well as, in some cases, to epithelial cells. The pattern of TIMP-2 expression was similar to that of MMP-2. We conclude that the stromal elements of ovarian tumors *express* MMP-2 and 9 and their specific inhibitors, but these do not seem to be controlled by endogenous TNF in the tumor microenvironment.

16/7/29 (Item 29 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07941825 94273351
Growth and major histocompatibility antigen expression regulation by IL-4, interferon-gamma (IFN-gamma) and tumour necrosis factor-alpha (TNF-alpha) on human renal cell carcinoma.
Hillman GG; Puri RK; Kukuruga MA; Pontes JE; Haas GP

Department of Urology, Wayne State University School of Medicine, Detroit, MI.
Clin Exp Immunol (ENGLAND) Jun 1994, 96 (3) p476-83, ISSN 0009-9104
Journal Code: DD7
Contract/Grant No.: CA 22453, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
We have recently shown that human renal cell carcinoma (RCC) tumour lines *express* high-affinity IL-4 receptors. Binding of IL-4 to RCC cells induced a growth inhibition in the range of 20-68%. To enhance the growth inhibitory effect of IL-4, we have tested the effects of two additional cytokines capable of directly affecting tumour cell growth. IFN-gamma caused a significant inhibition of RCC tumour cell growth (up to 70%) in a dose-dependent manner, whereas the effect of TNF-alpha was more limited (0-20% inhibition). The addition of IL-4 to IFN-gamma on RCC cells sensitive to IL-4 induced a greater inhibition of cell growth than that seen with each cytokine alone. IL-4 and IFN-gamma rendered RCC cells more responsive to the *inhibitory* effect mediated by *TNF* -alpha. The combination of TNF-alpha with IL-4 and IFN-gamma induced an optimal growth inhibition (up to 90-98%) of RCC cells. In addition to a direct anti-proliferative effect, we have demonstrated that these cytokines can also enhance the expression of MHC antigens on the surface of RCC tumour cell lines which may render the cells more immunogenic. All RCC lines tested expressed class I antigens, but not class II antigens. IFN-gamma induced class II expression and up-regulated the expression of class I antigens on RCC cells. Class II antigen expression was detectable following 48 h incubation, and greater after 72 h with IFN-gamma. IL-4 minimally affected class I expression, whereas TNF-alpha up-regulated class I antigen expression. IL-4 or TNF-alpha did not induce class II expression. The combination of the three cytokines slightly augmented the up-regulation of class I and class II antigens observed with IFN-gamma alone. These observations confirm the direct interaction of IL-4, IFN-gamma and TNF-alpha with RCC tumour cells, both at the level of growth regulation and MHC antigen expression, and suggest a therapeutic potential of the combination of the three cytokines for renal cell carcinoma.

07899868 94214133
Tumor necrosis factor (TNF)-induced protein phosphorylation in a human rhabdomyosarcoma cell line is mediated by 60-kD TNF receptors (TR60).
Mire-Sluis A; Meager A
Division of Immunobiology, National Institute for Biological Standards and Control, Potters Bar, Herts, UK.
Blood (UNITED STATES) Apr 15 1994, 83 (8) p2211-20, ISSN 0006-4971
Journal Code: A8G
Languages: ENGLISH
Document type: JOURNAL ARTICLE
In the present study, we used a cloned derivative, KYM-1D4, of the human rhabdomyosarcoma cell line, KYM-1, known to *express* high numbers of the two tumor necrosis factor (TNF) receptors, TR60 and TR80, and to be highly sensitive to TNF alpha-mediated cytotoxicity/antiproliferation, to investigate the role of TR60 and TR80 in protein phosphorylation. Using permeabilized KYM-1D4 cells, it was found that TNF alpha strongly induced phosphorylation of proteins of molecular weight 80, 65, 58, 42, and 30 kD. Addition of a monoclonal antibody (MoAb) against TR60 was shown to induce cytotoxicity/antiproliferation in KYM-1D4 cells and the same pattern of

protein phosphorylation as TNF alpha, whereas addition of an MoAb against TR80 was both noncytotoxic and ineffective in inducing protein phosphorylation. In contrast, in a highly TNF alpha-resistant KYM-1-derived cell line, 37B8R, no protein phosphorylation was induced with either TNF alpha or the agonistic anti-TR60 MoAb. However, when 37B8R was allowed to revert to partial TNF sensitivity by culture in the absence of TNF alpha, the resultant cell line, 37B8S, was found to regain inducibility of protein phosphorylation by TNF alpha. These results indicate that expression of functional TR60 in KYM-1-related cell lines is principally involved in TNF-mediated cytotoxicity/antiproliferation and is necessary for the induction of protein phosphorylation. Nevertheless, the latter, although apparently strongly associated with cytotoxicity, was probably involved in protective mechanisms because protein kinase C *inhibitors* that inhibited *TNF* alpha and anti-TR60-induced phosphorylation increased the cytotoxic/antiproliferative response to these mediators.

16/7/31 (Item 31 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07887957 94193682
Enhanced synthesis of *tumor* *necrosis* *factor*-inducible proteins, plasminogen activator *inhibitor* -2, manganese superoxide dismutase, and protein 28/5.6, is selectively triggered by the 55-kDa tumor necrosis factor receptor in human melanoma cells.
Smith DM; Tran HM; Soo VW; McQuiston SA; Tartaglia LA; Goeddel DV; Epstein LB
Cancer Research Institute, University of California, San Francisco 94143-0128.
J Biol Chem (UNITED STATES) Apr 1 1994, 269 (13) p9898-905, ISSN 0021-9258 Journal Code: HIV
Contract/Grant No.: CA 4446, CA, NCI; CA 27903, CA, NCI; AG08938, AG, NIA
Languages: ENGLISH
Document type: JOURNAL ARTICLE
We have demonstrated that A375 melanoma cells express mRNA for both types of tumor necrosis factor (TNF) receptors and receptor proteins on their plasma membranes. Specific agonist and blocking antibodies to either 55-kDa (TNF-R1) or 75-kDa (TNF-R2) TNF receptors combined with two-dimensional gel analysis were employed to determine which receptor type is responsible for mediating the induction of individual melanoma proteins. Our results indicate that the enhanced synthesis of proteins 21/>7 (M(r)/pI), 28/5.6, and 41/5.7 is selectively induced through TNF-R1. TNF induces these proteins; antagonist antibody to TNF-R1 prevents their induction by TNF, and TNF-R1 agonist induces them in the absence of TNF. Identification of these proteins by immunoblot analysis proved that 21/>7 is manganese superoxide dismutase, protein 28/5.6 is unrelated to 27/28-kDa heat shock protein, and protein 41/5.7 is plasminogen activator *inhibitor* -2. Furthermore, *TNF* cytotoxicity for A375 cells is also mediated by TNF-R1. These studies indicate that TNF-R1 is a critical signaling receptor for TNF action on A375 cells and demonstrate the potential use of TNF-R1 antibodies to selectively block or enhance specific effects of TNF on melanoma cells.

16/7/32 (Item 32 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07833664 94168479
The role of nitric oxide in parasitic diseases.

Liew FY
Department of Immunology, University of Glasgow, U.K.
Ann Trop Med Parasitol (ENGLAND) Dec 1993, 87 (6) p637-42, ISSN 0003-4983 Journal Code: 68E
Languages: ENGLISH
Document type: JOURNAL ARTICLE; REVIEW; REVIEW, TUTORIAL
Murine macrophages *express* high levels of nitric oxide synthase and produce large amounts of nitric oxide (NO) when stimulated with certain cytokines in the presence of a trace amount of lipopolysaccharide (LPS). The stimulatory cytokines include interleukin-1 (IL-1), interferon-gamma (IFN-gamma), *tumour* *necrosis* *factor*-alpha (*TNF*-alpha) and migration *inhibitory* factor. Activated macrophages are highly effective killers of intra- and extra-cellular pathogens. However, as excessive NO can lead to immunopathology (diabetes, graft-v.-host disease, EAE, liver cirrhosis, rheumatoid arthritis), NO production is necessarily under tight regulation. A number of cytokines, including IL-4, IL-10 and transforming growth factor-beta, can down regulate the induction of NO synthase in macrophages. In addition, macrophages exposed to LPS alone and then stimulated with a mix of IFN-gamma and LPS *express* significantly lower levels of NO synthase than cells stimulated without pre-exposure to LPS. Furthermore, NO can reduce the activity of NO synthase by feedback inhibition, and also inhibit the production of IFN-gamma by Th1 cells (thus turning off its own synthesis from upstream). The regulatory pathways involve tyrosine kinase and protein kinase C. (20 Refs.)

16/7/33 (Item 33 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07821886 93300801
Evidence for an acute phase response in human intestinal epithelial cells.
Molmenti EP; Ziambaras T; Perlmutter DH
Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri 63110.
J Biol Chem (UNITED STATES) Jul 5 1993, 268 (19) p14116-24, ISSN 0021-9258 Journal Code: HIV
Contract/Grant No.: DK45085, DK, NIDDK; T32 HD07409, HD, NICHD
Languages: ENGLISH
Document type: JOURNAL ARTICLE
During the host response to inflammation/tissue injury there are many changes in intermediary metabolism including a dramatic change in the concentrations of many "acute phase" plasma proteins. Although many of these acute phase proteins are predominantly derived from the liver and the response can be elicited from liver cells incubated in tissue culture with cytokines such as interleukin-6 (IL-6), interleukin-1 (IL-1), *tumor* *necrosis* *factor*-alpha, interferon-gamma, leukemia *inhibitory* factor, interleukin-11 (IL-11), and oncostatin M, there is now evidence that the response can also be elicited in extrahepatic tissues and cell types. In this study, we show that many of the acute phase plasma proteins are expressed in human intestinal epithelial cell lines Caco2 and T84 and that their expression is induced or regulated by cytokines IL-6, IL-1, interferon, and tumor necrosis factor in a manner characteristic of the acute phase response. In fact, effects of IL-1 and IL-6 which are additive, synergistic, and antagonistic in liver cell lines are also observed in these intestinal epithelial cell lines. Responses to IL-6 and IL-1 are seen at all stages of differentiation of Caco2 cells from crypt-like enterocytes to villus-like enterocytes. Caco2 cells *express* binding sites for IL-6 at both poles, for IL-1 at the basolateral pole and, to a lesser extent, at

the apical pole. T84 cells have IL-1 and IL-6 receptor binding sites only at the basolateral pole. IL-6 and IL-1 also regulate the expression of enterocyte-specific integral membrane proteins as exemplified by down-regulation of sucrase-isomaltase gene expression in response to IL-6. These data raise the possibility that enterocytes are involved in a local response to injury/inflammation at the epithelial surface and establish a model system for examining coordination of the acute phase response in a bipolar cell.

16/7/34 (Item 34 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07771968 94196896
[Expression of TNF receptors in rheumatoid arthritis and ankylosing spondylitis]
Expression von TNF-Rezeptoren bei rheumatoider Arthritis und Spondarthropathien.
Heilig B; Pezzutto A; Lukoschek M; Hunstein W
Medizinische Klinik und Poliklinik V. Universitat Heidelberg.
Z Rheumatol (GERMANY) Nov-Dec 1993, 52 (6) p383-9, ISSN 0340-1855 Journal Code: Y0V
Languages: GERMAN Summary Languages: ENGLISH
Document type: JOURNAL ARTICLE English Abstract
Tumor necrosis factor is an important mediator of the pathophysiologic events in synovitis. The expression of the p75 and p55-TNF-receptors in rheumatic diseases was investigated. Synovial mononuclear cells (SMNC) of patients with rheumatoid arthritis and spondylarthropathies *express* p75 TNF receptors in all cases, whereas SMNC of patients with traumatic synovitis do not. In 4/9 patients with rheumatoid arthritis and in 6/11 patients with spondylarthropathies SMNC also expressed the p55 TNF receptor. Differential analysis of lymphocytes and monocytes/macrophages revealed that both predominantly expressed the p75 *TNF* *receptor*. The highest concentrations of both *soluble* *TNF* *receptors* which may act as *TNF* antagonists were found in synovial fluids of rheumatoid arthritis patients.

16/7/35 (Item 35 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07634724 93123765
Regulation of HIV-1 expression by cytokine networks in a CD4+ model of chronic infection.
Butera ST; Roberts BD; Folks TM
Retrovirus Diseases Branch, Centers for Disease Control, Atlanta, GA 30333.
J Immunol (UNITED STATES) Jan 15 1993, 150 (2) p625-34, ISSN 0022-1767 Journal Code: IFB
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Using the CD4+ model of chronic HIV-1 infection, OM-10.1, we investigated the influence of TNF-alpha regulatory networks on induced viral expression. Previously, OM-10.1 cultures were characterized to respond to exogenous TNF-alpha, as nearly 100% of the cells were activated to *express* HIV-1 within 24 h. In this study, OM-10.1 cells were pulse-treated, by applying exogenous factors for short periods of time and then washing, to determine if autocrine TNF-alpha could sustain HIV-1 activation in the absence of

additional exogenous stimulation. After a TNF-alpha pulse treatment, the progressive increase of HIV-1-expressing OM-10.1 cells was prevented by the continuous presence of anti-TNF-alpha mAb. The inductive activity of supernatant from TNF-alpha pulse-treated OM-10.1 cultures was completely removed by absorption on immobilized anti-TNF-alpha mAb. In addition, TNF-alpha pulse-treated OM-10.1 cells activated HIV-1 expression in untreated OM-10.1 cells when cultured across a permeable membrane indicating paracrine effects. Interestingly, if TNF-alpha pulse-treated OM-10.1 cells were further pulse-treated with anti-TNF-alpha mAb, a marked reduction in autocrine TNF-alpha was observed although the level of newly synthesized TNF-alpha mRNA remained unaffected. A similar degree of inhibition over autocrine *TNF*-alpha production was observed when *soluble* *TNF* *receptors* were used as the second pulse treatment in these experiments. Although the applicability of these results to in vivo chronically HIV-1-infected cells remains to be realized, these results do indicate that activated HIV-1 expression can be influenced by self-perpetuating mechanisms during periods of limited exogenous stimulation. Furthermore, physiologic mechanisms involving soluble cytokine receptors that counteract autocrine and paracrine activation of HIV-1 expression are shown here to play a regulatory role.

16/7/36 (Item 36 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07608697 93357468
Regulation of manganese superoxide dismutase and other antioxidant genes in normal and leukemic hematopoietic cells and their relationship to cytotoxicity by tumor necrosis factor.

Kizaki M; Sakashita A; Karmakar A; Lin CW; Koeffler HP
Department of Medicine, Keio University, Tokyo, Japan.

Blood (UNITED STATES) Aug 15 1993, 82 (4) p1142-50, ISSN 0006-4971 Journal Code: A8G
Contract/Grant No.: CA33936, CA, NCI; DK41936, DK, NIDDK; CA26038, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Myeloid cells are a major source of superoxide and other oxygen metabolites. As a protective mechanism, cells *express* antioxidant enzymes including manganese superoxide dismutase (Mn-SOD), copper-zinc SOD (Cu/Zn-SOD), and glutathione peroxidase (GSX-PX). Even though hematopoietic cells are a major source of oxidants, little is known of their expression of antioxidants. We found that seven myeloid leukemic cell lines blocked at different stages of differentiation constitutively expressed Mn-SOD, Cu/Zn-SOD, and GSX-PX RNAs. Level of Mn-SOD activities paralleled levels of Mn-SOD RNA. Terminal differentiation of native HL-60 cells to either granulocytes or macrophages did not alter levels of Mn-SOD RNA but markedly decreased cell division. Myeloid leukemic lines sensitive to cytotoxic effects of tumor necrosis factor (TNF) as well as normal peripheral blood lymphocytes and monocytes, dramatically increased their levels of Mn-SOD RNA in the presence of TNF. In contrast, Cu/Zn-SOD and GSX-PX RNA levels did not increase in these same cells. TNF-resistant leukemic lines had higher constitutive levels of Mn-SOD RNA and activity; and these levels did not change in the presence of TNF. Antisense but not random oligonucleotides to Mn-SOD markedly increased the sensitivity to the *inhibitory* effects of *TNF* for both the native HL-60 (TNF-sensitive) and K562 (TNF-resistant) cell lines. Further studies showed that the antisense oligonucleotides entered the cells and resulted in decreased levels of Mn-SOD RNA. The data suggest that Mn-SOD may provide protection against

cytotoxicity of TNF in hematopoietic cells.

16/7/37 (Item 37 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07564152 93289864
Production of leukemia inhibitory factor mRNA and protein by malignant and immortalized bone cells.
Marusic A; Kalinowski JF; Jastrzebski S; Lorenzo JA
Veterans Administration Medical Center, Newington, Connecticut.
J Bone Miner Res (UNITED STATES) May 1993, 8 (5) p617-24, ISSN 0884-0431 Journal Code: 130
Contract/Grant No.: AR31263, AR, NIAMS; AR38933, AR, NIAMS
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Leukemia inhibitory factor (LIF) is a recently characterized glycoprotein with complex biologic activities on bone cells. We tested various rodent and human immortalized and malignant bone cell lines and primary osteoblast-enriched cell cultures from fetal rat calvarial digests for expression of LIF mRNA and LIF protein. Both human and rodent immortalized and malignant cells expressed a single 4.4 kb mRNA transcript that hybridized to a human LIF cDNA probe in Northern blots. LIF mRNA was undetectable in unstimulated rodent osteoblast-like cells lines MC3T3-E1 and Py1a. However, treatment with LPS (10 micrograms/ml), TGF-beta (1 ng/ml), *TNF*-alpha (100 ng/ml) or *inhibitors* of protein synthesis (cycloheximide, emetine, puromycin, and anisomycin) induced the expression of LIF message in these cells. In contrast, primary osteoblast-enriched cells did not *express* LIF mRNA in Northern blot assays either constitutively or after treatment with TNF-alpha or cycloheximide. The human osteosarcoma cells lines U-2 OS and Saos-2 constitutively expressed LIF mRNA and did not respond to LPS treatment. However, phorbol myristate acetate (PMA), an activator of protein kinase C, was a potent stimulator of LIF message in Saos-2 but not U-2 OS cells. The effects of PMA (0.5 ng/ml) on LIF mRNA in Saos-2 cells were detectable at 1 h and maximal at 6 h. *TNF*-alpha (100 ng/ml) and *inhibitors* of protein synthesis also increased LIF mRNA in both Saos-2 and U-2 OS cells. LIF protein was also detected constitutively in the conditioned medium from both Saos and U-2 OS cells.(ABSTRACT TRUNCATED AT 250 WORDS)

16/7/38 (Item 38 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07410476 92113256
Expression of a monocyte chemotactic cytokine by human mononuclear phagocytes.
Colotta F; Borre A; Wang JM; Tattanelli M; Maddalena F; Polentarutti N; Peri G; Mantovani A
Centro Catullo e Daniela Borgomainerio, Istituto di Ricerche Farmacologiche Mario Negri, Milano, Italy.
J Immunol (UNITED STATES) Feb 1 1992, 148 (3) p760-5, ISSN 0022-1767 Journal Code: IFB
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The present study was designed to investigate the capacity of human mononuclear phagocytes to produce a cytokine chemotactic for monocytes (monocyte chemotactic protein (MCP), alternative acronyms JE, monocyte

chemotactic and activating factor, MCP-1, and tumor-derived chemotactic factor). Human PBMC exposed in vitro to bacterial LPS expressed high levels of MCP transcripts. Monocyte-depleted lymphoid cells were not induced to *express* MCP by LPS. Percoll-gradient purified monocytes were able to *express* high levels of MCP transcripts. In an effort to exclude a role of contaminating non-monocytic cells, mononuclear phagocytes were separated by flow cytometry and sorting: CD14+ cells exposed to LPS showed high levels of MCP mRNA. LPS-stimulated monocytes released chemotactic activity for monocytes that could be inhibited by absorption with anti-MCP antibodies. IL-1, TNF, IFN-gamma, granulocyte-macrophage-CSF and, to a lesser extent, macrophage-CSF, as well as inactivated streptococci, also induced MCP gene expression. Actinomycin D experiments indicated that induction of MCP in monocytes was gene transcription-dependent. The protein synthesis *inhibitor* cycloheximide (Cy) blocked IL-1-, *TNF*-, or LPS-induced MCP gene expression in monocytes. In contrast, expression of the structurally related chemotactic cytokine IL-8 was superinduced by Cy. Moreover, Cy superinduced MCP gene expression in cells other than monocytes, including endothelial cells, smooth muscle cell and fibrosarcoma cells, indicating different mechanisms of regulation in mononuclear phagocytes vs cells of other lineages. The capacity of cells of the monocyte-macrophage lineage to produce a cytokine that recruits and activates circulating monocytes may be of considerable importance in inflammatory and immunologic reactions. Thus, the mononuclear phagocyte system can autonomously regulate the extravasation and activation of immature elements of the same lineage, a key event in inflammation and immunity.

16/7/39 (Item 39 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07138417 92282613
Expression of cytokine genes, cytokine receptor genes, and transcription factors in cultured Hodgkin and Reed-Sternberg cells.
Gruss HJ; Brach MA; Drexler HG; Bonifer R; Mertelsmann RH; Herrmann F
Department of Internal Medicine I, University of Freiburg Medical Center, Germany.
Cancer Res (UNITED STATES) Jun 15 1992, 52 (12) p3353-60, ISSN 0008-5472 Journal Code: CNF
Languages: ENGLISH
Document type: JOURNAL ARTICLE
In the present study, we show by Northern blot analysis and enzyme linked immunosorbent assay that the Hodgkin's disease (HD)-derived cell lines HDLM-2 and KM-H2 *express* a variety of cytokine genes either constitutively or upon induction with phorbol ester 12-O-tetradecanoylphorbol-13-acetate. Cytokine genes expressed by HD-derived lines include granulocyte-macrophage colony-stimulating factor (CSF), macrophage-CSF, interleukin (IL)-1-alpha, IL-3, IL-5, IL-6, IL-8, leukemia *inhibitory* factor, *tumor* *necrosis* *factor*-alpha, *tumor* *necrosis* *factor* -beta, and transforming growth factor-beta, while transcripts and the corresponding proteins for granulocyte-CSF, IL-1-beta, IL-2, IL-4, IL-7, IL-10, and the JE/macrophage chemoattractant and activating factor gene were not detectable in cytoplasmic RNA and culture supernatants obtained from both lines. In addition, IL-2 receptor (R) p55 and macrophage-CSF R (c-fms) genes were expressed by both lines. HDLM-2, but not KM-H2 cells, exhibited the IL-6 R p80 and the IL-2 R p75 chain. Analysis of nuclear proteins that bind to oligonucleotides containing the consensus sequences of the transcription factors activation protein 1, nuclear factor (NF) kappa B, and NFAT 1 revealed a pattern for HD lines resembling that of activated T-cells: HDLM-2 and KM-H2 cells constitutively

expressed NF binding to the NF of activated T-cells (type 1), previously described to be T-cell specific. In addition, NF kappa B-binding proteins obtained from both lines showed, in electrophoretic mobility shift assays, the same migration pattern as T-cell-derived proteins but differed from monocyte- and B-cell-derived proteins. UV cross-linking experiments confirmed that NF kappa B-binding proteins of M(r) 85,000, 75,000, and 50,000/55,000 were detectable in nuclear extracts obtained from T-cells and both HD lines, while monocytes and B-cells displayed the M(r) 50,000/55,000 and 75,000 NF kappa B complex only. Both HD lines also constitutively expressed transcripts for c-fos and c-jun, which are involved in heterodimeric formation of the transcription factor activation protein 1, as well as for the NF kappa B/KBF1 gene.

16/7/40 (Item 40 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07069549 92115723
Constitutive activity of the tumor necrosis factor promoter is canceled by the 3' untranslated region in nonmacrophage cell lines; a trans-dominant factor overcomes this suppressive effect.
Kruys V; Kemmer K; Shakhov A; Jongeneel V; Beutler B
Howard Hughes Medical Institute, Dallas, TX 75235-9050.
Proc Natl Acad Sci U S A (UNITED STATES) Jan 15 1992, 89 (2) p673-7,
ISSN 0027-8424 Journal Code: PV3
Contract/Grant No.: 5RO1-CA45525, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The role of the mouse tumor necrosis factor (TNF) promoter, 5' untranslated region (UTR), and 3' UTR in TNF gene expression has been examined in three nonmacrophage cell lines (HeLa, NIH 3T3, and L-929). The TNF promoter is not macrophage-specific. On the contrary, it constitutively drives reporter gene expression in all three cell lines. Not only the full-length promoter but also truncated versions of the promoter, lacking NF-kappa B binding motifs, are active in each type of cell. The TNF 3' UTR effectively cancels reporter gene expression in HeLa cells and in NIH 3T3 cells but fails to block expression in L-929 cells. L-929 cells contain a factor that overcomes the *inhibitory* influence of the *TNF* 3' UTR. Its action depends upon the presence of sequences found in the TNF 5' UTR. Cell-fusion experiments reveal that this activator is trans-dominant. These studies highlight the essential role played by the TNF 3' UTR, which silences the TNF gene in cells that might otherwise *express* TNF. They also reveal the existence of an escape mechanism whereby inappropriate synthesis of TNF might occur.

16/7/41 (Item 41 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

07061287 92118453
Chemoattractant(s) in culture supernatants of HTLV-I-Infected T-cell lines.
Saggioro D; Wang JM; Sironi M; Luini W; Mantovani A; Chieco-Bianchi L
Istituto di Oncologia, Centro Interuniversitario per la Ricerca sul Cancro (C.I.R.C.), I.S.T., Padova, Italy.
AIDS Res Hum Retroviruses (UNITED STATES) Jul 1991, 7 (7) p571-7,
ISSN 0889-2229 Journal Code: ART
Languages: ENGLISH

Document type: JOURNAL ARTICLE

Supernatants obtained from four HTLV-I transformed cell lines (MT2, MT4, C91/PL, and 81-66/45) induced in vitro migration of monocytes, polymorphonuclear leukocytes (PMN), and lymphocytes. The MT2, C91/PL, and 81-66/45 cell lines expressed both lymphotoxin (LT) and tumor necrosis factor (TNF-alpha) mRNA transcripts, and had TNF biological activity. In contrast, the MT4 cells did not *express* LT mRNA, had low levels of TNF-alpha transcript, and no TNF activity in the supernatant. Anti-TNF-alpha MAb, which blocks the chemotactic activity of recombinant *TNF*-alpha, had no *inhibitory* effect on the induction of migration by the MT2 and MT4 supernatants. Hence, no correlation was evident between TNF and chemotactic activity in supernatants of different HTLV-I-infected cell lines. Upon fractionation on Sephadex G50, the monocyte chemoattractant(s) eluted with two peaks in the 8-12 kD region, a size compatible with the chemotactic cytokines IL-8 and monocyte chemotactic protein (MCP). However, anti-IL-8 and anti-MCP antibodies did not have any effect, and Northern blot analysis showed that HTLV-I-transformed cell lines did not *express* mRNA transcripts of either IL-8 and MCP. These results demonstrate that HTLV-I transformed T-cell lines produce chemoattractant(s) active on PMN and monocytes, distinct from LT, TNF-alpha, IL-8, and MCP. Production of chemoattractants may play a role in the pathogenesis of diseases associated with HTLV-I infection.

16/7/42 (Item 42 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06982693 91122152

Inhibition of tumor necrosis factor-alpha transcription by Epstein-Barr virus.

Gosselin J; Menezes J; D'Addario M; Hiscott J; Flamand L; Lamoureux G; Oth D

Department of Microbiology and Immunology, University of Montreal, Quebec, Canada.

Eur J Immunol (GERMANY) Jan 1991, 21 (1) p203-8, ISSN 0014-2980
Journal Code: EN5

Languages: ENGLISH

Document type: JOURNAL ARTICLE

Tumor necrosis factor-alpha (TNF-alpha), which is produced mainly by monocyte/macrophage cells, has diverse physiological functions on lymphoid cells. Moreover, it has been shown that TNF-alpha exhibits antiviral activities. Here we report that Epstein-Barr virus (EBV), a B lymphotropic human herpes virus that interacts intimately with the immune system, exerts a strong *inhibitory* effect on *TNF* -alpha production by lipopolysaccharide-treated peripheral blood leukocytes as well as by monocytic cell lines, HL-60 and U-937. Flow cytometric analysis following staining with OKB7 monoclonal antibody showed that about 20% of cells from these monocytic lines *express* the CR2 antigen. Direct binding of fluorescein isothiocyanate-labeled EBV indicated that the virus binds to approximately 22% of cells of both monocytic lines. However, no virus-specific antigens were detected in the infected cells by immunofluorescence, suggesting that the infection was of the abortive type. The use of UV- or heat-inactivated EBV and *inhibitory* effect on *TNF* -alpha synthesis. These results suggest that infectious virus is necessary to obtain such an *inhibitory* effect. Analysis of *TNF*-alpha mRNA by polymerase chain reaction amplification indicated that the EBV suppressive effect is manifested at the transcriptional level. In contrast, EBV did not inhibit interleukin 1 mRNA production by these cells. These results indicate that EBV interacts directly with monocytes/macrophages to exert

its immunomodulatory effect.

16/7/43 (Item 43 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06721678 91208416
Effect of tumor necrosis factor-alpha on the proliferation of leukemic cells from children with B-cell precursor-acute lymphoblastic leukemia (BCP-ALL): studies of primary leukemic cells and BCP-ALL cell lines.
Zhou MX; Findley HW; Ma LH; Zaki SR; Hill T; Hamid M; Hooper WC; Ragab AH
Division of Pediatric Hematology/Oncology, Emory University, Atlanta, GA 30322.
Blood (UNITED STATES) May 1 1991, 77 (9) p2002-7, ISSN 0006-4971
Journal Code: A8G
Contract/Grant No.: CA20549, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
The effect of recombinant tumor necrosis factor-alpha (rTNF-alpha) on the primary leukemic blasts and leukemic cell lines derived from children with B-cell precursor acute lymphoblastic leukemia (BCP-ALL) was studied. The proliferation of leukemic cells from the bone marrow of 11 of 13 patients (seven at diagnosis, four in relapse) and from the 697 (BCP-ALL) cell line was significantly inhibited by rTNF-alpha at the lowest dose tested (0.1 ng/mL), as measured by 3H-TdR uptake. The degree of inhibition was variable, ranging from 17% to 78%. Furthermore, a dose-dependent inhibitory effect was observed, with approximately 70% mean inhibition of DNA synthesis detected when cells from 12 of 13 patients were incubated with 100 ng/mL of rTNF-alpha for 3 days. In contrast, rTNF-alpha did not inhibit another BCP-ALL cell line (EU-1/ALL) established recently in our laboratory. Studies indicated that the TNF-alpha gene was expressed by the primary leukemic blasts of one TNF-resistant case in his third relapse and by EU-1 cells. Also, TNF-alpha protein was detected by Western blot analysis and enzyme-linked immunoabsorbent assay in the supernatant of EU-1 cells; this is the first report of TNF production by a BCP-ALL cell lines. The production of TNF-alpha mRNA and protein was not detected in the 697 cell line and in the primary leukemic blasts from six patients (four at diagnosis, two in relapse) whose leukemic cells were inhibited by TNF. The partially purified TNF-alpha obtained from the EU-1 cell line also suppressed the proliferation of TNF-sensitive primary leukemic cells, and this inhibitory activity was abolished by an anti-TNF-alpha specific antibody. Our results demonstrate that *TNF*-alpha is an *inhibitor* of in vitro proliferation of BCP-ALL cells from most patients. The TNF-resistant leukemic cells from a few patients and the EU-1 cell line *express* TNF mRNA, suggesting that the induction of TNF gene expression is associated with the development of TNF resistance.

16/7/44 (Item 44 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06562150 91145932
Common expression of a tumor necrosis factor resistance mechanism among gynecological malignancies.
Powell CB; Mutch DG; Massad LS; Kao MS; Collins JL
Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, MO 63110.
Cancer Immunol Immunother (GERMANY) 1990, 32 (2) p131-6, ISSN

0340-7004 Journal Code: CN3
Languages: ENGLISH
Document type: JOURNAL ARTICLE

The efficacy of tumor necrosis factor alpha (TNF alpha) as an anticancer agent is limited. This limitation might be related to the expression of a protein-synthesis-dependent resistance mechanism that prevents the lysis of tumor cells by TNF alpha. To test this possibility eight randomly selected human cell lines, three derived from ovarian carcinomas and five derived from cervical carcinomas, were tested for their in vitro sensitivity to TNF alpha-mediated lysis. The results of this analysis showed that all eight cell lines are normally resistant to lysis by TNF alpha. However, in the presence of inhibitors of protein synthesis, seven of them showed a significant increase in TNF alpha-mediated lysis. Measurement of protein synthesis showed that there is a linear correlation between the level of inhibition of protein synthesis and the level of TNF alpha-mediated lysis. The fact that seven of eight randomly selected cell lines are resistant to TNF alpha because they *express* a protein-synthesis-dependent resistance mechanism suggests that this mechanism of resistance may be common among gynecological cancers. The results also suggest that a therapy involving *TNF* alpha and *inhibitors* of protein synthesis might be useful for the treatment of gynecological malignancies.

16/7/45 (Item 45 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06357274 90171856
A novel addition to the T cell repertory. Cell surface expression of tumor necrosis factor/cachectin by activated normal human T cells.

Kinkhabwala M; Sehajpal P; Skolnik E; Smith D; Sharma VK; Vlassara H; Cerami A; Suthanthiran M

Rogosin Institute, Department of Medicine, Cornell University Medical College, New York, New York 10021.

J Exp Med (UNITED STATES) Mar 1 1990, 171 (3) p941-6, ISSN 0022-1007 Journal Code: I2V
Contract/Grant No.: AI-21359, AI, NIAID; 5T35 AG-00086-09, AG, NIA
Languages: ENGLISH
Document type: JOURNAL ARTICLE

Expression of the pluripotent molecule TNF in a focused and antigen-restricted fashion might provide an advantage to the host organism. Given the central role of T cells in antigen-specific immunity, we examined whether activated T cells *express* TNF on their cell surface. FACS analysis of highly purified normal human T cells labeled with an anti-TNF mAb revealed that T cells *express* cell surface TNF when signaled with the synergistic combination of a calcium ionophore, ionomycin, and a protein kinase C activator, 12-o-tetradecanoyl phorbol acetate. Cell surface radioiodination studies of stimulated T cells demonstrated the presence of 26-kD transmembrane protein, a size predicted by TNF cDNA and different from that of the 17-kD secreted TNF molecule. The induced cell surface expression of TNF could be blocked with cyclosporine and/or methylprednisolone, and Northern analysis for *TNF*-specific transcripts revealed that this *inhibitory* effect occurs pretranslationally. Our demonstration for the first time that stimulated normal human T cells display cell surface TNF provides a mechanistic basis for the realization of effects of TNF in an antigen-specific fashion.

16/7/46 (Item 46 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

06239757 88115998
Tumor necrosis factor expression in human epithelial tumor cell lines.
Spriggs DR; Imamura K; Rodriguez C; Sariban E; Kufe DW
Laboratory of Clinical Pharmacology, Dana-Farber Cancer Institute, Boston, Massachusetts 02115.
J Clin Invest (UNITED STATES) Feb 1988, 81 (2) p455-60, ISSN 0021-9738 Journal Code: HS7
Contract/Grant No.: CA00994, CA, NCI
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Tumor necrosis factor (TNF) is a monokine with in vitro cytotoxicity for some but not all tumor cells. The basis for sensitivity and resistance to the antitumor effects of this agent remains unclear. The present studies have monitored the effects of TNF on 14 epithelial tumor cell lines. Eleven of these cell lines were resistant to the growth *inhibitory* effects of *TNF* (50% *inhibitory* concentration greater than 1,000 U/ml). 12 of the 14 tumor cell lines has detectable levels of high affinity cell surface TNF binding sites, thus suggesting that resistance was not often due to the absence of cell surface TNF receptors. Northern blot analysis demonstrated that three of the eleven resistant cell lines expressed detectable levels of TNF mRNA. Furthermore, both sensitive and resistant epithelial tumor cells had the capacity to *express* TNF transcripts in the presence of the protein synthesis *inhibitor*, cycloheximide. Finally, the presence of *TNF* expression at the RNA level is shown to be associated with the production of a TNF-like protein in the resistant Ov-D ovarian carcinoma cells. These findings suggest that certain human epithelial tumor cell lines inherently resistant to TNF also *express* this cytokine.

16/7/47 (Item 47 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05843559 89292015
Regulation of levels of IL-1 mRNA in human fibroblasts.
Yamato K; el-Hajjaoui Z; Koeffler HP
Department of Medicine, UCLA School of Medicine 90024.
J Cell Physiol (UNITED STATES) Jun 1989, 139 (3) p610-6, ISSN 0021-9541 Journal Code: HNB
Contract/Grant No.: CA26038, CA, NCI; CA33936, CA, NCI; CA43277, CA, NCI; +
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Interleukin-1 (IL-1) has a crucial role in host defenses, inflammatory processes, and tissue homeostasis. A wide variety of cells produce this protein in response to a number of extracellular stimuli including microorganisms, antigenic stimuli, and products from other cells. Regulation of IL-1 production at the molecular level is poorly understood. We studied expression, intracellular signals, and posttranscriptional regulation of IL-1 mRNA in human mesenchymal cells by using Northern blot analysis. Tumor necrosis factor alpha (TNF alpha) and activators of protein kinase C including 12-O-tetradecanoylphorbol-13-acetate (TPA) and teleocidin induced the accumulation of IL-1 beta mRNA in human fibroblasts (WI-38). Effect of *TNF* alpha was not blocked by *inhibitors* of either protein synthesis (cycloheximide) or protein kinase C activity. Accumulation of IL-1 beta mRNA was also increased by a calcium ionophore (A23187) and an inhibitor of the Na+/K+ pump (ouabain); both compounds are known to increase cytoplasmic levels of Ca++. Stability of IL-1 beta mRNA

in fibroblasts exposed to TPA was more than fourfold greater than after fibroblasts were exposed to either TNF alpha or cycloheximide. This suggests that posttranscriptional stabilization of IL-1 beta mRNA is a major mechanism leading to accumulation of IL-1 beta mRNA after activation of PKC in fibroblasts. Fibroblasts did not *express* IL-1 alpha mRNA after exposure to stimuli which induced the accumulation of IL-1 beta mRNA. In summary, several different pathways regulate levels of IL-1 beta mRNA in human mesenchymal cells.

16/7/48 (Item 48 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05715601 90063082
Tumor necrosis factor mediates autocrine growth inhibition in a chronic leukemia.
Duncombe AS; Heslop HE; Turner M; Meager A; Priest R; Exley T; Brenner MK
Department of Haematology, Royal Free Hospital, London, U.K.
J Immunol (UNITED STATES) Dec 1 1989, 143 (11) p3828-34, ISSN 0022-1767 Journal Code: IFB
Languages: ENGLISH
Document type: JOURNAL ARTICLE
Autocrine production of growth factors may contribute to the rapid and fatal proliferation of acute hematologic malignancies. We have investigated whether the more controlled growth of less aggressive malignancies such as chronic myeloid leukemia (CML) may be associated with autocrine production of growth *inhibitory* factors. *TNF* inhibits the growth of both normal and leukemic hemopoietic progenitor cells. We find that exogenous TNF reduces the viability and DNA synthesis of purified myeloid cells from patients with CML and inhibits myeloid colony formation by patient progenitor cells. However, unlike progenitor cells from normal donors, patient myeloid progenitor cells also constitutively *express* mRNA for TNF and secrete functional TNF protein in culture. This endogenous TNF impedes the growth of CML cells because anti-TNF mAb shown to neutralize bioactive human TNF increases CML cell DNA synthesis whereas non-neutralizing anti-TNF mAb has no effect. Production of TNF by CML cells is not associated with production of lymphotoxin (TNF-beta), IL-1 or IL-6. TNF-mediated autocrine growth inhibition may contribute to the maintenance of the stable, chronic phase of this disease and similar mechanisms may operate in other malignancies to limit tumor proliferation. Competition between autocrine growth promoting and inhibiting factors may underlie the observed differences in biologic behavior between acute and chronic malignancies.

16/7/49 (Item 49 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05677842 89080232
Human vascular smooth muscle cells. Target for and source of tumor necrosis factor.
Warner SJ; Libby P
Department of Medicine, Tufts University, Boston, MA 02111.
J Immunol (UNITED STATES) Jan 1 1989, 142 (1) p100-9, ISSN 0022-1767 Journal Code: IFB
Contract/Grant No.: HL-34636, HL, NHLBI
Languages: ENGLISH
Document type: JOURNAL ARTICLE

TNF-alpha (also known as cachectin) may produce many of its important effects in vivo by actions on blood vessels. Endothelial cells are well known to respond to TNF-alpha. We investigated whether vascular smooth muscle cells (SMC), the most abundant cell type in most vessels, also respond to TNF-alpha and the related cytokine lymphotoxin (TNF-beta). Both human rTNF-alpha and beta (0.1 to 100 ng/ml) induced transient accumulation of IL-1 mRNA by adult human vascular SMC that peaked between 1 and 4 h. The inhibitor of RNA synthesis actinomycin D (1 microgram/ml) blocked the induction of IL-1 mRNA, whereas inhibition of protein synthesis with cycloheximide (1 microgram/ml) resulted in a marked "superinduction" of both IL-1 alpha and IL-1 beta mRNA species. TNF-alpha treatment also increased intracellular biologically active IL-1 and subsequent release of IL-1 activity from SMC. Metabolic labeling and immunoprecipitation with specific antibodies demonstrated de novo synthesis of IL-1 alpha and IL-1 beta precursors in TNF-treated or lymphotoxin-treated SMC. TNF-alpha also activated other SMC functions including the concentration-dependent release of PGE2 from SMC, and time-dependent induction of the gene for (2'-5')-oligoadenylate synthetase, an enzyme thought to mediate the anti-viral and anti-proliferative actions of IFN. We also explored whether SMC, which both produce and respond to IL-1, might also express either of the TNF genes. Bacterial LPS (10 micrograms/ml) caused slight accumulation of TNF-alpha transcripts. Incubation of SMC for 4 h with inhibitors of protein synthesis alone caused little or no elevation of TNF-alpha mRNA, but simultaneous addition of LPS ("superinduction" conditions) induced large amounts of TNF-alpha (but not TNF-beta) mRNA. Cells treated with anisomycin (1 microgram/ml) and LPS, then washed to remove this reversible *inhibitor* of protein synthesis, released *TNF*-alpha into the medium, as assessed by the L929 cytotoxicity assay and by metabolic labeling and immunoprecipitation. Thus, SMC both respond to both TNF and lymphotoxin and can produce TNF-alpha, a cytokine with numerous effects on vascular cells of potential significance in the pathophysiology of septic shock and other inflammatory conditions.

16/7/50 (Item 50 from file: 155)
DIALOG(R)File 155:MEDLINE(R)

05342962 88168698
Gene cloning and structure--function relationship of cytokines such as TNF and interleukins.

Fiers W; Beyaert R; Brouckaert P; Everaerdt B; Haegeman G; Suffys P; Tavernier J; Vandenabeele P; Vanhaesebroeck B; Van Ostade X; et al
Laboratory of Molecular Biology, University of Gheni, Belgium.

Immunol Lett (NETHERLANDS) Dec 1987, 16 (3-4) p219-26, ISSN 0165-2478 Journal Code: GIH
Languages: ENGLISH
Document type: JOURNAL ARTICLE

The genes for a number of proteins, potentially useful in cancer therapy and collectively called "biological response modifiers", have been cloned and expressed in micro-organisms in recent years. These recombinant proteins, which are now available in pure form in nearly unlimited quantities, include interferons, interleukins and cytotoxins such as Tumor Necrosis Factor (TNF) and lymphotoxin. Most often the human gene has been cloned and expressed, with view to possible applications in medicine, but usually the mouse equivalent gene was also characterized in order to carry out syngeneic animal model experiments. TNF is selectively toxic for many transformed cell lines, either alone or in combination with interferon or inhibitors of RNA or protein synthesis. Cells sensitive to the cytotoxic action of TNF and cells unaffected by it nonetheless usually carry about an

equal number of TNF receptors; hence it is the secondary, intracellular signal which makes the difference between a transformed cell and a normal, diploid cell. TNF can induce a number of different genes in a variety of cells; for example, endothelial cells *express* a surface antigen responsible for adherence of leucocytes. Another gene which is induced by TNF is interleukin 6 (also called 26 kDa protein or BSF-2). This interleukin, IL-6, is a growth and differentiation factor for B cells as well as for T cells; it is responsible for functions previously ascribed to hepatocyte-stimulating factor, but has no interferon activity. The toxic action of TNF on tumor cells must involve the release of arachidonic acid as phospholipase *inhibitors* block the *TNF*-induced effects.(ABSTRACT TRUNCATED AT 250 WORDS)

16/7/51 (Item 1 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

13185690 BIOSIS Number: 99185690
Nitrogen oxide in host defense against parasites
Oswald I P; James S L
INRA, Lab. Pharmacol.-Toxicol., 180 chemin de Rournefeuille, 31391 Toulouse Cedex, France
Methods (Orlando) 10 (1). 1996. 8-14.
Full Journal Title: Methods (Orlando)
ISSN: 1046-2023
Language: ENGLISH
Print Number: Biological Abstracts Vol. 102 Iss. 008 Ref. 117697
Despite its small size and transitory nature, nitric oxide (NO) is a very versatile molecule. In addition to its function as a potent vasodilator and neurotransmitter, NO is important in inflammation and immunity. Indeed, in vitro experiments demonstrated that NO production by cytokine-activated rodent cells is a primary mediator of their antimicrobial and antitumoral activity. NO results from the oxidative deimination of L-arginine to L-citrulline by NO synthase (NOS), several isoforms of which have recently been isolated. Numerous cells types produce high levels of NO as a result of the expression of the inducible NO synthase (INOS) after stimulation with bacterial product and/or cytokines, leading to parasite elimination. iNOS activity is highly regulated by cytokines, with some of them acting to induce enzyme expression (IFN-gamma, *TNF*-alpha), and others acting as *inhibitory* cytokines (TGF-beta, IL-4, IL-10, and IL-13). While a strong correlation between antiparasitic activity and NO production by cytokine-activated cells has been readily demonstrated in vitro, the relationship between generation of NO in vivo and protection against parasitic infection has only recently been addressed. Although human cells such as hepatocytes have been shown to *express* iNOS, the presence of such a pathway in human monocyte/macrophages is a subject of great controversy.

16/7/52 (Item 2 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

11723685 BIOSIS Number: 98323685
Fas antigen expression of CD34+ human marrow cells is induced by interferon gamma and tumor necrosis factor alpha and potentiates cytokine-mediated hematopoietic suppression in vitro
Maciejewski J; Selleri C; Anderson S; Young N S
Build. 10, Room 7C103, Natl. Inst. Health Bethesda, MD 20892, USA
Blood 85 (11). 1995. 3183-3190.

Full Journal Title: Blood
ISSN: 0006-4971
Language: ENGLISH
Print Number: Biological Abstracts Vol. 100 Iss. 003 Ref. 031409

Activation of Fas antigen, a cell surface receptor molecule, by its ligand results in transduction of a signal for cell death. The Fas system has been implicated in target cell recognition, clonal development of immune effector cells, and termination of the cellular immune response. Fas antigen expression on lymphocytes is regulated by interferon gamma (IFN-gamma) and tumor necrosis factor alpha (*TNF*-alpha), cytokines that also have *inhibitory* effects on hematopoiesis. We investigated Fas antigen expression on human marrow cells and the effects of Fas activation on hematopoiesis in vitro. Freshly isolated immature hematopoietic cells, as defined by the CD34 marker, did not *express* Fas antigen at levels detectable by fluorescent staining. CD34+ cells, which include progenitors and stem cells, showed low levels of Fas expression in culture, even in the presence of growth factors. Stimulation by TNF-alpha and IFN-gamma markedly increased Fas antigen expression on CD34+ cells. Anti-Fas antibody, which mimics the action of the putative ligand, enhanced IFN-gamma- and TNF-alpha-mediated suppression of colony formation by bone marrow (BM) in a dose-dependent manner. This effect did not require the presence of accessory cells. Colony formation from mature (CD34+ CD38+) and immature (CD34+CD38-) progenitor cells and long-term culture initiating cells were susceptible to the inhibitory action of anti-Fas antibody in the presence of IFN-gamma and TNF-alpha. Apoptosis assays performed on total BM cells and CD34+ cells showed that anti-Fas antibody induced programmed cell death of CD34+ BM cells. Fas antigen may be expressed as part of the differentiation program of hematopoietic cells. Fas antigen and its ligand may play a role in the pathophysiology of marrow failure states and in the elimination of abnormal hematopoietic cells in the course of an immune response.

16/7/53 (Item 3 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

11025827 BIOSIS Number: 97225827
Positive and negative selection for *tumor* *necrosis* *factor* responsiveness reveals an *inhibitory* role for EGF receptor in *TNF* -induced antiproliferation

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Lymphokine and Cytokine Research 13 (1). 1994. 37-45.
Full Journal Title: Lymphokine and Cytokine Research
ISSN: 1056-5477
Language: ENGLISH
Print Number: Biological Abstracts Vol. 097 Iss. 010 Ref. 142935

Tumor necrosis factor (TNF) induces dose-dependent, but incomplete cytotoxicity in ME-180 cervical carcinoma cells resulting in a significant reduction in cell viability. In this cell line there exists a characteristic residual tumor cell population that appears to be resistant to TNF. In order to investigate tumor cell heterogeneity and characteristics that correlate with their escape from TNF-induced cytotoxicity, TNF-resistant ME-180 cell variants (ME-180R) were isolated from a population of ME-180 cervical carcinoma cells (ME-180 parental). Incubation of ME-180 parental cells with TNF resulted in measurable changes in tumor cell DNA structural integrity and dose-dependent cytotoxicity,

whereas ME-180R cell growth and DNA integrity were not effected by incubation with TNF. Binding of 125I-labeled TNF to a TNF-specific cell-surface receptor was measurable and equivalent on both ME-180R and ME-180 parental cells and both cell lines predominantly expressed the p55 form of the TNF receptor based upon flow cytometric analysis. Although both cell lines shared similar doubling times, intrinsic EGF receptor tyrosine kinase activity in ME-180R cells was found to be gt 3-fold higher than that isolated from ME-180 parental cells. These results suggest that TNF-responsiveness may be mediated at a point subsequent to TNF binding and may be regulated, in part, by the expression of tyrosine kinase activity. To further explore this hypothesis, A431 vulvular carcinoma cells that *express* resistance to TNF were cloned and variants were isolated that escaped EGF-induced growth inhibition. These variants were all shown to *express* 2- to 4-fold lower levels of EGF receptor protein or intrinsic tyrosine kinase activity when compared to the parental A431 population and coordinately expressed sensitivity to the growth *inhibitory* effects of *TNF* . These results suggest that EGF receptor may function to suppress TNF-induced growth inhibition or cytotoxicity in squamous carcinoma cells. Modulation of EGF receptor levels or intrinsic tyrosine kinase activity may represent one mechanism whereby tumor cells escape TNF-induced antiproliferation.

16/7/54 (Item 4 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

10967434 BIOSIS Number: 97167434
Expression of TNF receptors in rheumatoid arthritis and spondylarthropathies
Heilig B; Pezzutto A; Lukoschek M; Hunstein W
Med. Klin. und Poliklin. V, Hospitalstr. 3, 69115 Heidelberg, GER
Zeitschrift fuer Rheumatologie 52 (6). 1993. 383-389.
Full Journal Title: Zeitschrift fuer Rheumatologie
ISSN: 0340-1855
Language: GERMAN
Print Number: Biological Abstracts Vol. 097 Iss. 008 Ref. 101106
Tumor necrosis factor is an important mediator of the pathophysiologic events in synovitis. The expression of the p75 and p55-TNF-receptors in rheumatic diseases was investigated. Synovial mononuclear cells (SMNC) of patients with rheumatoid arthritis and spondylarthropathies *express* p75 TNF receptors in all cases, whereas SMNC of patients with traumatic synovitis do not. In 4/9 patients with rheumatoid arthritis and in 6/11 patients with spondylarthropathies SMNC also expressed the p 55 TNF receptor. Differential analysis of lymphocytes and monocytes/macrophages revealed that both predominantly expressed the p75 *TNF* *receptor*. The highest concentrations of both *soluble* *TNF* *receptors* which may act as *TNF* antagonists were found in synovial fluids of rheumatoid arthritis patients.

16/7/55 (Item 5 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

10872634 BIOSIS Number: 97072634
Expression, regulation, and production of tumor necrosis factor-alpha in mouse testicular interstitial macrophages in vitro
Xiong Y; Hales D B
Dep. Physiol. Biophysics, Univ. Illinois Chicago, 901 South Wolcott Ave.,

Room E-202, Chicago, IL 60612-7342, USA
Endocrinology 133 (6). 1993. 2568-2573.
Full Journal Title: Endocrinology
ISSN: 0013-7227
Language: ENGLISH
Print Number: Biological Abstracts Vol. 097 Iss. 004 Ref. 040139

Tumor necrosis factor-alpha (TNF-alpha) is a cytokine principally secreted from macrophages and monocytes activated by agents such as lipopolysaccharide (LPS). We have recently shown that TNF-alpha inhibited mouse Leydig cell steroidogenesis in vitro. LPS injection has also been shown to repress Leydig cell function and induce TNF-alpha messenger RNA (mRNA) expression in testicular interstitial macrophages in vivo. A paracrine regulation of Leydig cell testosterone synthesis by testicular interstitial macrophages via TNF-alpha has been proposed. To further support this possibility, we examined whether LPS can induce TNF-alpha mRNA expression and protein production in testicular interstitial macrophages in vitro. The regulation of LPS-stimulated TNF-alpha mRNA expression in vitro was also investigated by employing the protein synthesis *inhibitor* cycloheximide (CHX). *TNF*-alpha secretion into culture supernatants was examined by both bioassay and enzyme-linked immunosorbent assay. Isolated testicular interstitial macrophages were cultured for 24 h before the initiation of treatments. Cells were treated with or without LPS (1.0 mu-g/ml) and in the presence or absence of CHX (5.0 mu-g/ml) at different time points. Northern blot analysis showed that TNF apprx mRNA was rapidly and significantly induced by LPS in testicular interstitial macrophages. The peak expression was at 2 h after the treatment, which was 8.3 +- 2.6-fold over the control (P lt 0.05). TNF apprx mRNA then declined quickly and completely disappeared by 8 h after LPS treatment. In contrast to this rapid and transient induction of TNF apprx message by LPS alone, CHX extended the induction and caused a marked increase in LPS-induced TNF-alpha mRNA at 2 and 6 h. CHX induced more LPS-stimulated TNF-alpha mRNA at 6 h than that at 2 h. At 3 h after LPS treatment, TNF-alpha secretion was significantly stimulated (5.6 +- 1.2 U/mu-g macrophage DNA) measured by L929 tumor fibroblast cytotoxicity. TNF-alpha was also detected by enzyme-linked immunosorbent assay in culture media of testicular interstitial macrophages treated with control medium or LPS for 1, 2, and 6 h. TNF-alpha secretion was increased in a time-dependent way. There are significantly higher LPS-induced TNF-alpha levels in culture media at 2 h (35.4 +- 2.2 pg/mu-g macrophage DNA) and 6 h (85.5 +- 11.1 pg/mu-g macrophage DNA) than those in control groups. The current study demonstrates that LPS activates testicular interstitial macrophages to *express* TNF-alpha mRNA and secrete TNF-alpha protein in vitro. CHX super-stimulates and extends this LPS-induced TNF-alpha mRNA expression, which indicates the existence of potential inhibitors in testicular interstitial macrophages that repress TNF-alpha mRNA expression. These results thus provide additional evidence to support the hypothesis TNF-alpha secreted from testicular interstitial macrophages exerts a paracrine regulation of Leydig cell steroidogenesis in the testis.

16/7/56 (Item 6 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

10500014 BIOSIS Number: 96100014
NOVEL INTERACTIONS BETWEEN DERMAL DENDROCYTES AND MAST CELLS IN HUMAN SKIN IMPLICATIONS FOR HEMOSTASIS AND MATRIX REPAIR
SUEKI H; WHITAKER D; BUCHSBAUM M; MURPHY G F
DEP. DERMATOL., UNIV. PENN. SCH. MED., 235B CLIN. RES. BUILD., 422 CURIE BLVD., PHILADELPHIA, PA 19104-6142, USA.

LAB INVEST 69 (2). 1993. 160-172. CODEN: LAINA
Full Journal Title: Laboratory Investigation
Language: ENGLISH

BACKGROUND: Dermal dendrocytes are a newly-recognized cell type in human skin. They *express* coagulation Factor XIIIa (FXIIIa), also known as fibrin stabilizing factor, and their number is increased in certain inflammatory dermatoses. Current dogma suggests that these recently described cells may represent a subset of antigen-presenting macrophages. The present study therefore was undertaken to examine further the phenotype and potential function of these novel cells. EXPERIMENTAL DESIGN: Conventional ultrastructure, single and dual label immunofluorescence and immunocytochemistry, confocal laser scanning microscopy, and immunoelectron microscopy were used to define structure and potential heterogeneity among dermal dendrocytes. Human neonatal foreskin organ culture exposed to mast cell secretagogues, *inhibitors*, and relevant recombinant cytokines (*tumor* *necrosis* *factor* a) was employed to gain insight into functional characteristics of FXIIIa expression. RESULTS: We found that dermal dendrocytes are phenotypically unique dermal cells, separate from conventional macrophages, with antigenic heterogeneity of FXIIIa and CD34 expression related to their microanatomical location in the dermis. Moreover, they *express* specialized membrane-matrix plaques that may stabilize their placement in various dermal strata. Finally, superficial subpopulations of dermal dendrocytes are closely-associated with mast cells and show enhanced FXIIIa expression in response to mast cell degranulation, an event that appears to result from liberation of mast cell tumor necrosis factor in the dendrocyte microenvironment. CONCLUSIONS: These new insights establish dermal dendrocytes as distinctive fixed skin cells with potential functional capacity for mast cell-dependent facilitation of fibrin cross-linking and matrix remodeling. These previously unrecognized phenotypic and functional characteristics of dermal dendrocytes therefore may be relevant to cellular interactions responsible for cutaneous wound healing and hemostasis.

16/7/57 (Item 7 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

8624120 BIOSIS Number: 92089120
CHEMOATTRACTANTS IN CULTURE SUPERNATANTS OF HTLV-I-INFECTED T-CELL LINES
SAGGIORO D; WANG J M; SIRONI M; LUINI W; MANTOVANI A; CHIECO-BIANCHI L
IST. ONCOL., VIA GATTAMELATA 64, 35128 PADOVA, ITALY.
AIDS RES HUM RETROVIRUSES 7 (7). 1991. 571-578. CODEN: ARHRE
Full Journal Title: Aids Research and Human Retroviruses
Language: ENGLISH

Supernatants obtained from four HTLV-I transformed cell lines (MT2, MT4, C91/PL, and 81-66/45) induced in vitro migration of monocytes, polymorphonuclear leukocytes (PMN), and lymphocytes. The MT2, C91/PL, and 81-66/45 cell lines expressed both lymphotoxin (LT) and tumor necrosis factor (TNF-.alpha.) mRNA transcripts, and had TNF biological activity. In contrast, the MT4 cells did not *express* LT mRNA, had low levels of TNF-.alpha. transcript, and no TNF activity in the supernatant. Anti-TNF-.alpha. MAb, which blocks the chemotactic activity of recombinant *TNF*-.alpha., had no *inhibitory* effect on the induction of migration by the MT2 and MT4 supernatants. Hence, no correlation was evident between TNF and chemotactic activity in supernatants of different HTLV-I-infected cell lines. Upon fractionation on Sephadex G50, the monocyte chemoattractant(s) eluted with two peaks in the 8-12 kD region, a size compatible with the chemotactic cytokines IL-8 and monocyte chemotactic protein (MCP). However, anti-IL-8 and anti-MCP antibodies did not have any effect, and Northern

blot analysis showed that HTLV-I-transformed cell lines did not *express* mRNA transcripts of either IL-8 and MCP. These results demonstrate that HTLV-I transformed T-cell lines produce chemoattractant(s) active on PMN and monocytes, distinct from LT, TNF-.alpha., IL-8, and MCP. Production of chemoattractants may play a role in the pathogenesis of diseases associated with HTLV-I infection.

16/7/58 (Item 8 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

7135373 BIOSIS Number: 88058118
REGULATION OF LEVELS OF IL-1 MESSENGER IN HUMAN FIBROBLASTS
YAMATO K; EL-HAJJAOUI Z; KOEFFLER H P
DEP. MED., DIV. HEMATOL./ONCOL., UCLA SCH. MED., LOS ANGELES, CALIF. 90024.
J CELL PHYSIOL 139 (3). 1989. 610-616. CODEN: JCLLA
Full Journal Title: Journal of Cellular Physiology
Language: ENGLISH
Interleukin-1 (IL-1) has a crucial role in host defenses, inflammatory processes, and tissue homeostasis. A wide variety of cells produce this protein in response to a number of extracellular stimuli including microorganisms, antigenic stimuli, and products from other cells. Regulation of IL-1 production at the molecular level is poorly understood. We studied expression, intracellular signals, and posttranscriptional regulation of IL-1 mRNA in human mesenchymal cells by using Northern blot analysis. Tumor necrosis factor alpha (TNF.alpha.) and activators of protein kinase C including 12-O-tetradecanoylphorbol-13-acetate (TPA) and teleocidin induced the accumulation of IL-1.beta. mRNA in human fibroblasts (WI-38). Effect of *TNF*.alpha. was not blocked by *inhibitors* of either protein synthesis (cycloheximide) or protein kinase C activity. Accumulation of IL-1.beta. mRNA was also increased by a calcium ionophore (A23187) and an inhibitor of the Na+/K+ pump (ouabain); both compounds are known to increase cytoplasmic levels of Ca++. Stability of IL-1.beta. mRNA in fibroblasts exposed to TPA was more than fourfold greater than after fibroblasts were exposed to either TNF.alpha. or cycloheximide. This suggests that posttranscriptional stabilization of IL-1.beta. mRNA is a major mechanism leading to accumulation of IL-1.beta. mRNA after activation of PKC in fibroblasts. Fibroblasts did not *express* IL-1.alpha. mRNA after exposure to stimuli which induced the accumulation of IL-1.beta. mRNA. In summary, several different pathways regulate levels of IL-1.beta. mRNA in human mesenchymal cells.

16/7/59 (Item 9 from file: 5)
DIALOG(R)File 5:BIOSIS PREVIEWS(R)

7071313 BIOSIS Number: 87131834
DYSCOORDINATE EXPRESSION OF TUMOR NECROSIS FACTOR-ALPHA BY HUMAN BLOOD MONOCYTES AND ALVEOLAR MACROPHAGES
RICH E A; PANUSKA J R; WALLIS R S; WOLF C B; LEONARD M L; ELLNER J J
PULMONARY DIV., DEP. OF MED., UNIV. HOSP. OF CLEVELAND, 2074 ABINGTON RD., CLEVELAND, OHIO 44106.
AM REV RESPIR DIS 139 (4). 1989. 1010-1016. CODEN: ARDSB
Full Journal Title: American Review of Respiratory Disease
Language: ENGLISH
Previous studies have shown that human alveolar macrophages produce less interleukin-1 (IL-1) in response to lipopolysaccharide (LPS) than do their

precursors, blood monocytes. The purpose of this study was to compare the capacities of alveolar macrophages and blood monocytes to synthesize tumor necrosis factor (TNF) in response to LPS. Alveolar macrophages were obtained by bronchoalveolar lavage of healthy nonsmoking subjects, and blood monocytes were obtained by adherence of mononuclear cells to plastic. TNF activity was measured in supernatants and cell lysates as cytotoxicity to L929 fibroblasts (uptake of neutral red at 570 nm). TNF activity of alveolar macrophages stimulated at 106 cells/ml with LPS (10 .mu.g/ml) for 16 h was 596 .+-. 367, and of blood monocytes it was 60 .+-. 84 U/ml (mean .+-. SD, p < 0.005). At no concentration of LPS and at no period of stimulation did alveolar macrophages *express* less TNF activity than did blood monocytes. In concurrent experiments, supernatants of LPS-stimulated alveolar macrophages contained less IL-1 activity than did blood monocytes. Lysates of both cell types contained <20% of total TNF activity. The TNF activity of LPS-stimulated alveolar macrophages was neutalized >99% by monoclonal antibody to TNF-alpha; control monoclonal antibody OKT3 had no effect. Next, alveolar macrophages and blood monocytes were biosynthetically labeled with [3H]leucine during incubation with LPS; supernatants were immunoprecipitated with anti-TNF; and precipitates were electrophoresed on polyacrylamide gels. Autoradiographys indicated that immunoreactive TNF was produced by both blood monocytes and alveolar macropohages and that the relative moleclar weights were identical (17,000). In vitro cultivation of blood monocytes for 1 to 10 days resulted in increased expression of TNF but not of IL-1 activity when cells were stimulated with LPS, suggesting that maturation of mononuclear phagocytes contributes to their differentiation to cells capable of producing higher levels of TNF. Because cytotoxicity and immunosuppression have been linked, the role of TNF produced by alveolar macrophages in suppression of lymphocyte responses to phytohemagglutinin was examined. The lymphocyte inhibitory activity was unaffected by the addition of sufficient monoclonal antibody to neutralize the contained TNF activity, and recombinant *TNF* had no direct *inhibitory* effect on lymphocyte responses to the mitogen. Thus, alveolar macrophages are potent producers of bioactive/immunoreactive TNF; TNF is not a mediator of the immunosuppressive activity of alveolar macrophages. Considering the multiple functions of TNF including tumor cell cytotoxicity, tissue necrosis, and induction of pulmonary edema, it is likely that regulation of TNF production in vivo is important for normal homeostasis as well as factor in the pathogenesis of certain lung diseases characterized by tissue destruction.

16/7/60 (Item 1 from file: 266)
DIALOG(R)File 266:FEDRIP

0285508
IDENTIFYING NO.: 5F32AR08391-02 AGENCY CODE: CRISP
GENE THERAPY FOR ARTHRITIS USING ANTI TNF-ALPHA
PRINCIPAL INVESTIGATOR: GHIVIZZANI, STEVEN C
ADDRESS: UNIVERSITY OF PITTSBURGH PITTSBURGH, PA 15261
PERFORMING ORG.: UNIVERSITY OF PITTSBURGH AT PITTSBURGH, PITTSBURGH, PENNSYLVANIA
SPONSORING ORG.: NAT INST OF ARTHRITIS AND MUSCULOSKELETAL AND SKIN DISEASES
FY : 96 FUNDS: $32,500 TYPE OF AWARD: Noncompeting Continuation (Type 5)
SUMMARY: Arthritis is the most frequently reported chronic condition among American females and the second most frequent overall. Certain cytokines such as TNF-alpha and interleukin-1 have been shown to be key mediators in the arthritic disease state. To overcome problems associated

with delivery of anti-arthritic agents, our laboratory has developed an ex vivo method for transferring genes encoding therapeutic proteins to the lining of the joint. This proposal is designed to evaluate the therapeutic efficacy of different inhibitors of TNF-alpha in blocking antigen induced arthritis when delivered to the joint by gene transfer. The specific aims are: 1) To construct retroviral vectors expressing monovalent and bivalent forms of TNF soluble receptors p55 and p75.2) To express the mono- and bivalent TNF-alpha soluble receptors intraarticularly in the rabbit knee by transplantation of genetically modified synoviocytes. 3) To determine the efficacy of each expressed TNF inhibitor in the treatment of antigen-induced arthritis in rabbit knee joints. 4) To determine the efficacy of coexpressing TNF-alpha inhibitors and interleukin-1 receptor antagonist protein in the treatment of antigen-induced arthritis in rabbit knee joints. The results of these experiments should lead to identification of the appropriate therapeutic protein(s) to be used in further clinical trials for treatment of arthritis by gene transfer.

16/7/61 (Item 2 from file: 266)
DIALOG(R)File 266:FEDRIP

0261315
IDENTIFYING NO.: 0097775; COLV05420 AGENCY CODE: AGRIC
BACTERIAL DISEASES AND THE IMMUNE SYSTEM
PRINCIPAL INVESTIGATOR: BAXTER G
ASSOCIATE INVESTIGATORS: HOWELL M; VANDEWOUDE S
PERFORMING ORG.: COLORADO STATE UNIVERSITY, MICROBIOLOGY, FORT COLLINS, COLORADO 80523
SPONSORING ORG.: U. S. DEPARTMENT OF AGRICULTURE, COOPERATIVE STATE RES SER
DATES: 921021 TO 970930
SUMMARY: OBJECTIVE: To continue development of new procedures for the rapid and reliable detection of the causative agents of bacterial diseases; to develop new, more efficacious methods for the prevention of diseases caused by bacterial agents; and to study the pathogenic processes and epidemiology of bacterial diseases.

APPROACH: Improved diagnostic procedures will be developed for the detection of several important pathogenic bacteria (for example, Brucella ovis; Mycobacterium ovis; mycobacterium paratuberculosis and clostridium perfringens). Tests will include improved enzyme immunoassays, polymerase chain reaction to detect bacterial DNA and/or RNA, and use of fluorescently labeled recombinant DNA probes and antibodies to specific coat proteins. These reagents will be used to study the disease process and the epidemiology of individual infections. Finally, in some cases, more efficacious recombinant vaccines will be developd to prevent the disease.

PROGRESS REPORT SUMMARY: PROGRESS: The potential for DNA based vaccines is being investigated.The gene for murine granulocyte-macrophage colony stimulating factor (GM-CSF) has been cloned and expressed in E. coli. The recombinant protein is being characterized for its ability to support the growth of a GM-CSF dependent cell line, to confirm that the cytokine is biologically active. In addition, the gene for murine soluble tumor necrosis factor receptor (sTNFR) has been isolated by PCR amplification, and the sTNFR gene was cloned into a prokaryotic expression construct. Murine sTNFR was expressed and secreted using the construct. Techniques are being developed to introduce and express genetic constructs in single animal organs. Cationic liposomes have been used to introduce a marker E. coli lacZ gene into the kidney of a rat by injection into the renal artery; expression of the lacZ marker gene within proximal tubules of the injected kidney, without any evidence of expression in lung, liver, spleen or

contralateral kidney. The molecular basis of alphaherpes virus persistence invertebrates was investigated using probes specific to the latency activated transcripts. One virus with a large deletion in the LAT region produced the initial viral transcripts and proteins required for replication of virus, but the virus was unable to complete replication. These data suggest a mechanism for LAT in the regulation of reactivation.

16/7/62 (Item 1 from file: 72)
DIALOG(R)File 72:EMBASE

10095036 EMBASE No: 96276068
Activation of adenosine A3 receptors on macrophages inhibits tumor necrosis factor-

McWhinney C.D.; Dudley M.W.; Bowlin T.L.; Peet N.P.; Schook L.; Bradshaw M.; De M.; Borcherding D.R.; Edwards III C.K.
Weis Center for Research, Geisinger Clinic, Danville, PA 17822 USA

European Journal of Pharmacology (Netherlands) , 1996, 310/2-3 (209-216)
CODEN: EJPHA ISSN: 0014-2999
LANGUAGES: English SUMMARY LANGUAGES: English

Murine macrophage-derived tumor necrosis factor alpha (TNF-alpha) gene expression has been shown to be dramatically induced by bacterial lipopolysaccharide, and to be dependent upon nuclear factor-kappaB (NF-kappaB) binding sites in its promoter for the lipopolysaccharide induction. Murine J774.1 macrophage cells were found to predominately *express* the adenosine A3 receptor RNA relative to adenosine A1 receptor or adenosine A2 receptor RNA. Adenosine receptor agonists, in a dose-dependent manner characteristic of the adenosine A3 receptor, blocked the endotoxin induction of the TNF-alpha gene and TNF-alpha protein expression in the J774.1 macrophage cell line. The adenosine A3 receptor antagonist BW-1433 dose-dependently reversed this adenosine *inhibitory* effect on *TNF* -alpha gene expression. Thus, the binding of adenosine receptor agonists to the adenosine A3 receptor interrupts the endotoxin CD14 receptor signal transduction pathway and blocks induction of cytokine TNF-alpha, revealing a novel cross-talk between the murine adenosine A3 receptor and the endotoxin CD14 receptor in J774.1 macrophages.

16/7/63 (Item 2 from file: 72)
DIALOG(R)File 72:EMBASE

9117650 EMBASE No: 94061234
NF-kappaB and IkappaBalpha: An inducible regulatory system in endothelial activation

Read M.A.; Whitley M.Z.; Williams A.J.; Collins T.
Department of Pathology, Brigham and Women's Hospital, 221 Longwood Avenue, Boston, MA 02115 USA

J. EXP. MED. (USA) , 1994, 179/2 (503-512) CODEN: JEMEA ISSN: 0022-1007
LANGUAGES: English SUMMARY LANGUAGES: English

Structural analysis of the promoters of several endothelial genes induced at sites of inflammatory or immune responses reveals binding sites for the transcription factor nuclear factor kappaB (NF-kappaB). Endothelial cells *express* transcripts encoding the p50/p105 and p65 components of NF-kappaB and the rel- related proto-oncogene c-rel; steady state levels of these transcripts are transiently increased by tumor necrosis factor alpha (TNF-alpha). Western blotting revealed that stimulation of endothelial cells with TNF-alpha resulted in nuclear accumulation of the p50 and p65

components of NF-kappaB. Ultraviolet crosslinking and immunoprecipitation demonstrated binding of the p50 and p65 components of NF-kappaB to the E-selectin kappaB site. Endothelial cells *express* an inhibitor of NF-kappaB activation, IkappaB-alpha (MAD-3). Protein levels of this *inhibitor* fall rapidly after *TNF*-alpha stimulation. In parallel, p50 and p65 accumulate in the nucleus and RNA transcript levels for IkappaB-alpha are dramatically upregulated. Recombinant p65 stimulates expression of E-selectin promoter-reporter constructs. IkappaB-alpha inhibits p65 or TNF-alpha-stimulated E- selectin promoter-reporter gene expression in transfected endothelial cells. The NF-kappaB and IkappaB-alpha system may be an inducible regulatory mechanism in endothelial activation.
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TO: M. Paul Barker (ATTORNEY, AGENT, FIRM OR AGENCY)

Finnegan Henderson (ATTORNEY'S DOCKET NUMBER OR APPLICATION NUMBER)

202 408-4400 (FAX/TELECOPIER NUMBER)

FROM: Garnette D. Draper, SPE

ART UNIT 1812 PHONE NUMBER: (703) 308-4232

Remarks: Attached is 326 cover letter, 892 listing the citations, and copies of Art that you may not be able to get quickly (a "V" is beside these on the 892). Any other items needed, please contact the SPE, Michelle Richardson. - Thanks Garnette Draper

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TO: M. Paul Barker
(ATTORNEY, AGENT, FIRM OR AGENCY)

Finnegan Henderson
(ATTORNEY'S DOCKET NUMBER OR APPLICATION NUMBER)

202 408-4400
(FAX/TELECOPIER NUMBER)

FROM: Garnette D. Draper, SPE

ART UNIT 1812 PHONE NUMBER: (703) 308-4232

Remarks: Attached is 326 cover letter, 892 listing the citations, and copies of Art that you may not be able to get quickly (a "Y" is beside them on the 892). Any other items needed please contact the SPE, Michelle Richardson. - Thank You Garnette D Draper

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DATE: 6/3/97

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TO: Paul Barker
(ATTORNEY, AGENT, FIRM OR AGENCY)

08/375242 & copy of action in 484337
(ATTORNEY'S DOCKET NUMBER OR APPLICATION NUMBER)

202 408-4400
(FAX/TELECOPIER NUMBER)

FROM: Garnette D. Draper, SPE

ART UNIT 1812 PHONE NUMBER: (703) 308-4232

Remarks: A copy of the office action you requested is attached (6 pages). I've also attached a copy of a recent action issued last week in S.N. 08/484337. This is the other case that I mentioned to you. Although you did not initially request an interview in it, since it is related, I suggested that you ~~[illegible]~~ possibly look at it (similar issues).

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